

Serial No.: 10/004,219

REMARKS

The application has been amended to include a Sequence Listing in conformity with 37 C.F.R. § § 1.821-1.825. Submitted herewith is the Sequence Listing, a copy of the Sequence Listing in computer readable form (CRF), as well as a Statement under 37 C.F.R. § § 1.821(f) and 1.825. Appendices A and B also include the Sequence Listing.

The application further has been amended to cancel drawing figures 2 and 8A (*see*, attached Proposed Amendment to Drawing Figures). The sequences that were contained in these drawing figures have been incorporated into the text of the specification. Drawing figure 8B has been amended to increase the font size of the sequences contained therein and now is presented as figure 8.

It is respectfully requested that no new matter has been added by the amendment. However, if any questions remain after consideration of the instant amendments, the Office is kindly requested to contact applicants' attorney at the address or telephone number given herein.

Respectfully submitted,



Tawni L. Wilhelm
Registration No. 47,456
Attorney for Applicant
TRASKBRITT, PC
P. O. Box 2550
Salt Lake City, Utah 84110-2550
Telephone: (801) 532-1922

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APPENDIX B

**(VERSION OF SUBSTITUTE SPECIFICATION EXCLUDING CLAIMS
WITH MARKINGS TO SHOW CHANGES MADE)**

(Serial No. 10/014,219)

PATENT
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APPLICATION FOR LETTERS PATENT

for

**A MAMMALIAN MUCINASE, ITS RECOMBINANT PRODUCTION, AND ITS USE
IN THERAPY OR PROPHYLAXIS AGAINST DISEASES IN WHICH MUCUS IS
INVOLVED OR INFECTIOUS DISEASES**

Inventors:
J.M.F.G. Aerts
R.G. Boot

Attorney:
Allen C. Turner
Registration No. 33,041
TRASKBRITT
P.O. Box 2550
Salt Lake City, Utah 84110
(801) 532-1922

TITLE OF THE INVENTION

A MAMMALIAN MUCINASE, ITS RECOMBINANT PRODUCTION, AND ITS USE IN THERAPY OR PROPHYLAXIS AGAINST DISEASES IN WHICH MUCUS IS INVOLVED OR INFECTIOUS DISEASES

TECHNICAL FIELD

[0001] The invention relates to the field of medicine. More specifically, the invention relates to therapeutic or prophylactic treatment of an individual against a disease in which mucus is involved and/or an infection disease. The invention also relates to the preparation of a mucinase suitable for the treatment.

BACKGROUND

[0002] Mucus as protective barrier. Mucins form part of the dynamic, interactive defensive system of mammals at mucosal surfaces in, for example, the gastrointestinal tract, the respiratory tract, and reproductive organs. Mucins are highly glycosylated proteins occurring either as secretory or membrane-bound forms. They have a unique molecular structure and chemical properties. The polypeptide backbone (apomucin) is rich in hydroxy amino acids, serine and threonine, which together with glycine, alanine and proline comprise nearly 50% of total amino acid residues of the protein and are present as tandemly repeated sequences. The threonine and serine residues are the targets of O-glycosylation machinery and the extent of glycosylation is such that carbohydrates account for 50-85% of the dry weight of mucins. Secretory mucins are the major constituents of mucus secretions, lining the epithelial cells of digestive, respiratory and reproductive tracts (Gendler et al., 1995, Gum 1995). They are capable of forming gels at very low concentration by forming long thread-like polymers resulting from the formation of disulphide linkages between monomers and intramolecular interactions of sugar side chains. Membrane-bound mucins are present on the surface of various cell types and, unlike secretory mucins, do not form oligomers and are hence smaller in size than their secretory counterparts (Gendler et al., 1995, Gum 1995). The membrane-bound mucins also have O-glycosylated serine and threonine-rich regions, but they lack tandem repeat sequences. The primary function of secretory mucins is to provide protection to the apical epithelial cell layers in digestive, respiratory and urinogenital tracts

against environmental factors like acidic pH, hydrolytic enzymes and pathogens. The cell surface mucins, in addition to their protective role, have a shielding effect on various surface receptors, thereby helping in the regulation of their activity (Strous and Dekker 1992). So far, twelve human mucin genes have been identified, designated as MUC1-4, MUC5AC, MUC5B, MUC6-9, and MUC11-12 (Gendler et al., 1995, Gum 1995, Gum et al., 1990, Lan et al., 1990, Moniaux et al., 1999, Shankar et al., 1997, Williams et al., 1999). They can be divided into secreted and membrane-associated forms, each with characteristic protein domains and tissue-specific glycosylation. Eight human mucin genes have been well characterized: MUC2, MUC5AC, MUC5B, MUC6 map to 11p15.5 and encode secretory gel-forming mucins while MUC1, MUC3, MUC4, MUC7 are scattered on different chromosomes and encode membrane-bound or secreted mucins.

[0003] Historically, purified mucins have been identified by their amino and carbohydrate composition consisting of a high percentage of serine, threonine, proline, alanine, glycine, and a large proportion of O-linked oligosaccharides (up to 80% of the total mass). Biosynthetic pathways have been described for the secreted and membrane-associated mucins and their eventual degradation and turnover. Mucins are present at all mucosal surfaces throughout the body in typical combinations and relate to the demands of organ function. Patterns of MUC gene expression with gastrointestinal site-specific glycosylation are clearly important but are not yet well defined. The mucosal surface throughout the gastrointestinal tract must resist the aggressive elements from the external environment present in the diet and encountered during normal function. This defensive system is based on fundamental characteristics shared with the barrier found at other mucosal surfaces. The stable protective barrier enabling exchange between the epithelial cells of the gut lumen for the purposes of nutrition and protection is made up of a layer of secreted mucus and a cell-surface membrane glycocalyx. The mucus defensive barrier forms the first line of defense to the external environment and contains both innate and adaptive immune elements.

[0004] Mucin expression. Mucins are present at all mucosal surfaces throughout the body. The specific functional requirements for mucus at each site are reflected in the appearance of different mucins in the cells at different sites in the body. The main population of specially adapted cells producing secreted mucins are the goblet cells. The proportion of goblet cells increases through the gastrointestinal tract (GI tract) with maximal numbers in the rectum. Goblet cells in the respiratory tract are present in the trachea and, to a lesser extent, in the bronchi. They are rarely found in bronchioles less than 1 mm in diameter

(Jeffery et al., 1992). Mucins have a tissue-specific glycosylation at each site in the gastrointestinal tract. As carbohydrate constitutes the major part of all mature mucins and is represented by vast arrays of different oligosaccharide structures, the potential for multiple functions related to bulk carbohydrate or individual structures must be examined. Most of the oligosaccharides in mucins are attached by O-links. However, a much smaller number of N-linked chains are also present, linked to asparagine residues in the mucin polypeptide through an N-glycosidic bond to N-acetyl-D-glucosamine. N-linked oligosaccharides contain a branched trimannosyl-chitobiose pentasaccharide core attached to the peptide.

[0005] Mucus degradation. The adherent mucus barrier and the glycocalyx are constantly being turned over as part of their protective functions at the mucosal surface. Thus, degradation of mucus is a normal feature of an equilibrium between mucosal synthesis, secretion and the breakdown of the existing adherent gel. This balance must be regulated to ensure continual mucosal protection against potentially damaging compounds and organisms entering in the diet. The first stage in mucus degradation is conversion of the mucus gel to a viscous fluid. The viscoelastic properties of the secreted, adherent gel layer are governed by the mucins, and they are responsible for the gel-forming properties. The non-mucin components of mucus may influence gel formation or affect gel strength. They are implicated in pathological situations where alterations in the normal composition of mucus are made. Mucinase activity of bacteria has been described and is well known from observations of mucin carbohydrate release, sequestration and metabolic conversion by bacteria in the large intestine. A population of mucin oligosaccharide-degrading (MOD) bacterial strains capable of specific and complete degradation of mucins has been identified. Bacterial mucinase enzymes have been shown to act on the mucus gel to reduce it to a viscous fluid, probably through the action of proteinases or peptidases, to act further on the accessible peptide backbone, not blocked by oligosaccharide substitution, and to cleave the individual sugars from the oligosaccharide chains. Bacterial mucinase activity has been measured in fecal extracts using electrophoretic assessment of mucin degradation and by direct mucinase assays with purified biotinylated mucins.

[0006] Mucin-associated diseases. Changes in mucus are frequent in inflammatory diseases of the epithelia. High levels of secretion of the mucin proteins is a common factor in, for instance, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), chronic bronchitis, asthma, tuberculosis, and carcinomas. Conversely, in Inflammatory Bowel Disease such as Crohns disease and ulcerative colitis, the mucus barrier

is decreased. Furthermore, mucus appears to play a role in infection by mucus-containing pathogens.

[0007] Mucus obstruction in Cystic Fibrosis. Cystic Fibrosis is the most common lethal genetic disorder among Caucasians. Presently, approximately 30,000 children, adolescents, and adults in the United States are affected by this disease, as reported on the Cystic Fibrosis Foundation Web site (www.cff.org). The median age of survival is 32.3 years of age.

[0008] A variety of symptoms, the most common ones being salty-tasting sweat and skin, persistent cough, wheezing, and failure to thrive (due to intestinal defects, malnutrition and anorexia), characterize the disease. The usual complications in the respiratory tract are: hemoptysis (blood in the sputum); pneumothorax (collapsed lung); atelectasis (air resorption leaving the lobe or segment airless) caused by complete mucus plugging; dilated bronchioles and bronchi and weakened bronchioles and bronchi walls; fibrosis (scar tissue); and low oxygen levels. Respiratory failure in CF is usually at the end of a long process where frequently there is no longer enough healthy lung tissue left to eliminate CO₂. It is widely believed that the respiratory sequela in CF and progressive deterioration of respiratory function are the result of persistent bacterial colonization (culminating with chronic *P. aeruginosa* infections) and chronic inflammation. The major cause of high morbidity and mortality in CF remains the chronic respiratory infections (most notably with *P. aeruginosa*) which account for more than 90 percent of CF mortality.

[0009] Cystic fibrosis (CF) is a multiorgan disease that is the result of a genetic defect of a single gene. The gene, CF transmembrane conductance regulator (CFTR), was identified in 1989 (Riordan et al., 1989). The gene encodes a membrane glycoprotein that functions as a cAMP-regulated chloride channel in exocrine glands and secretory epithelia.

[0010] As a result, thick and adhesive mucus is present in the airways and gastrointestinal tract of cystic fibrosis patients, leading to respiratory symptoms, recurrent infections, and progressive lung destruction, as well as nutritional deficiencies. The viscosity of CF mucus is determined by the presence of mucins. In addition, purulent mucus of CF patients contains as much as 3-14 mg/ml DNA (Chernick and Barbero 1959, Potter et al., 1960). This DNA, derived from inflammatory cells and epithelial cells, contributes (together with actin) to the viscosity of purulent CF sputum. Therefore, mucus transport by mucociliary activity and/or cough is hampered. In addition, the viscous DNA-containing mucus may also

cause reduced effectiveness of aminoglycoside antibiotics. These two aspects of CF mucus viscosity result in persistent, recurring infections and progressive lung destruction.

[0011] As the pathogenesis of cystic fibrosis is complex, treatment for CF consists of several approaches. The pulmonary disease is managed by combinations of physiotherapy, antibiotics (especially to contain *Pseudomonas aeruginosa* infections, which is an important cause of death in CF patients), mucolytics (n-acetylcysteine, recombinant human DNase I), bronchodilators and anti-inflammatory agents such as oral corticosteroids. The nutritional support mainly consists of the administration of pancreatic enzyme preparations to help food digestion, which is hampered by obstruction of the pancreatic ducts.

[0012] The aerosol route can be used to deliver mucoactive medications locally. These mucoactive medications comprise mucolytics, mucokinetic agents, mucoregulatory medications and expectorants and ion channel modifiers.

[0013] Several studies have focused on the use of recombinant human DNase (rhuDNase) I to reduce the viscosity of cystic fibrosis sputum. In *in vitro* assays, the viscosity of purulent CF sputum as well as its adhesiveness was shown to decrease after treatment with recombinant human DNase I (Pulmozyme®, Genentech), and the mucociliary transportability of CF sputum is increased (Shak et al., 1990, Zahm et al., 1995). This is due to the degradation of DNA but also to the depolymerization of F-actin (Vanscelllos et al., 1994) Phase 3 clinical trials have shown that treatment with aerosolized recombinant human DNase I results in a 28-37% reduction in respiratory exacerbations and an improvement of 5.6-5.8% in FEV1, a measure for lung function (Fuchs et al., 1994, Shak et al., Chest 1995).

[0014] COPD. Chronic Obstructive Pulmonary Disease (COPD) is a physiologically defined group of conditions characterized by the presence of persistent airflow obstruction. COPD is defined as a disease state characterized by the presence of airflow obstruction due to chronic obstructive bronchitis or emphysema.

[0015] One of the hallmarks of bronchitis is hyperproduction of mucus as well as loss of mucociliary clearance. Even though an effect of rhuDNase has been shown to reduce chronic bronchitis sputum viscosity (Puchelle et al., 1996), mucus in chronic bronchitis contains 10-fold less DNA than in CF sputum (Kim et al., 2001). This indicates that Pulmozyme, which is a human DNase that is used to reduce viscosity of CF sputum, is not equally effective as a mucolytic for bronchitis mucus. Chronic bronchitis is currently treated with bronchodilators, β -adrenergic agents, methylxanthines, corticosteroids, and mucolytics,

mostly with n-acetylcysteine. N-acetylcysteine has mucolytic activity *in vitro*; this activity, however, has not been demonstrated convincingly *in vivo* (Celli et al., 1995)

[0016] Asthma. In chronic asthma, as in CF and bronchitis, decreased mucociliary clearance caused by mucus hypersecretion and/or rheological changes and permanent changes in ciliary structure and function occur. Airway inflammation plays a major role in the pathophysiology of asthma. Standard asthma treatment comprises antihistamines, bronchodilators, leukotrien inhibitors, and (gluco-)corticosteroids.

[0017] Tuberculosis. Another disease in which airway mucus viscosity is increased and acetylcysteine is used as a mucolytic is pulmonary tuberculosis. Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*, which is transmitted by aerosols of saliva and mucus released by coughing. Pulmonary tuberculosis is associated with persistent cough and expectoration of bloody mucus. The disease is generally treated by long-term therapy with combinations of antibiotics.

[0018] Intensive care medicine (respiration). Patients in intensive care that are attached to a respirator also have problems clearing airway mucus. The airways of these patients need to be cleared regularly to prevent stasis and opportunistic infections. To this aim, these patients get antibiotics prophylactically.

[0019] Carcinoma. Mucins are thought to promote tumor-cell invasion and metastasis. In many human carcinomas, the expression profile of mucins is altered, with certain mucins like MUC1 being upregulated while others show a downregulated expression. The glycosylation process is disrupted in cancer, leading to aberrantly glycosylated, mostly underglycosylated mucins. In gastric carcinomas, the alterations of the mucin expression have been the subject of several studies. The expression of MUC5AC, a secretory mucin present in normal gastric mucosa, is downregulated and can be found in only 60% of the intestinal carcinomas. At the same time, the expression of MUC1 and MUC2 in gastric carcinomas was upregulated. The decrease in the amount of glycosylation of MUC1 with the progression of carcinogenesis was shown with a panel of antibodies binding with different affinities to glycosylated and unglycosylated forms of MUC1. Furthermore, in cancer cells the expression of MUC1 was distributed over the entire cell membrane, while it was limited to the apical region of normal gastric mucosa cells.

[0020] The expression pattern of the mucin genes is complex in normal airways involving six genes, mainly MUC5AC and MUC5B in mucus-producing cells and MUC4 in a wide array of epithelial cells. MUC5AC overexpression in metaplasia, dysplasia and normal

epithelium adjacent to squamous cell carcinoma provides additional arguments for a mucous cell origin of preneoplastic squamous lesions. MUC5AC and MUC5B expression is related to mucus formation in adenocarcinomas. Mucinous bronchiolalveolar carcinoma (BAC) has a particular pattern of mucin gene expression indicating that it has sustained a well-differentiated phenotype similar to the goblet cell, correlated with distinctive features, i.e., a noninvasive pattern and a better prognosis than nonBACs. MUC4 is the earlier mucin gene expressed in the foregut, before epithelial differentiation, and is expressed independently of mucus secretion both in normal adult airways and carcinomas. These findings are in favor of the histogenetic theory of non-small-cell carcinoma originating from a pluripotent mucous cell.

[0021] Several arguments suggest that mucins play a role in tumor-cell invasion and metastasis, resulting in prognostic implications. MUC1 is a transmembrane molecule with a large extracellular domain protruding high above the cell surface thought to reduce cell-cell and extracellular matrix (ECM)-cell adhesion in cancer cells (Jentoft 1990, Hudson et al., 1996) but direct evidence for a role of specific mucin genes in tumor progression is lacking. One study shows that splenic-portal inoculation in athymic mice of MUC2 antisense construct in highly metastatic human colon cancer cells resulted in a reduction in MUC2 levels and a marked decrease in liver colonization (Sternberg et al., 1999). Sialomucin complex (SMC), a rat homologue of the human mucin MUC4 isolated from highly metastatic ascites 13762 mammary adenocarcinoma cells is thought to potentiate metastasis by sterical disruption of molecular interactions for cell-cell and cell-ECM adhesions and by suppression of anti-tumor immunity by inhibition of interactions between cytotoxic lymphocytes and target tumor cells (Carraway et al., 2000). One recent study shows that *in vivo*, subcutaneous injection of SMC-overexpressing cells results in substantially greater lung metastasis than injection of SMC-repressed cells. Moreover, injection of A375 human melanoma cells followed by *in vivo* induction of SMC overexpression within the solid tumor resulted in spontaneous distant metastasis (Komatsu et al., 2000).

[0022] Mucus-containing pathogens. Mucins and mucin-like molecules have recently been described in several protozoan parasites, at different stages of the life cycle. These include kinetoplastid *Trypanosoma*, *Leishmania*), apicomplaxan (*Cryptosporidium*) and amoebic (*Entamoeba*) parasites (Schenkman et al., 1993, Almeida et al., 1994, Ilg et al., 1999, Barnes et al., 1998, Strong et al., 2000). These share many structural and compositional features with mammalian mucins, but vary in several other aspects. It is now becoming

evident that mucins in a parasite are involved in cell-cell interaction and cell surface protection, thus helping the parasite to establish infection.

[0023] Currently, several pharmaceutical compounds against diseases in which mucus is involved are used. Those compounds have, however, limited beneficial effects. A major reason for this is the fact that the targets of therapeutic compounds, like, for instance, lung epithelial cells, can hardly be reached because of the barrier of thick and adhesive mucus which is present in the airways and gastrointestinal tract of the patient. After administration of a certain pharmaceutical composition, only a small percentage of the composition is actually capable of performing its beneficial effect. Higher doses often do not improve treatment; essentially no more targets can be reached. Furthermore, higher doses often lead to more harmful side effects. A major part of administered pharmaceutical composition therefore often leaves the body before any beneficial effect could be performed.

[0024] The incapability of current therapeutic compounds to reach their target efficiently is a major drawback of current treatment.

[0025] Mucus thus provides an important defensive barrier which forms the first line of defense to the external environment. However, several diseases involve a disturbed generation of mucus, resulting in thick and adhesive mucus. This mucus forms an unwanted barrier hampering the uptake of, for instance, nutrition and/or medicines by a patient. This is, for instance, a major problem for patients suffering from cystic fibrosis, COPD, asthma, bronchitis, and tuberculosis. Uptake of nutrients in the gastrointestinal tract is insufficient because of the decreased permeability of the mucus layer covering the epithelial cells. This often results in failure to thrive. Likewise, pharmaceutical compositions are less able to reach their target, for instance, in the lungs, because of a thick mucus layer. This reduces the efficiency of current treatment. Therefore, there is a need for a means of decreasing the mucus barrier in a mammal.

SUMMARY OF THE INVENTION

[0026] The present invention provides a recombinant or substantially isolated or purified mammalian mucinase, or a modified form thereof having a substantially similar mucin-hydrolyzing activity. The invention also provides a recombinant or substantially isolated or purified mucinase, the mucinase being a mucinase having an amino acid sequence essentially corresponding to the amino acid sequence shown in ~~FIG-8~~ Table I below (SEQ ID

NO:1), or a modified form of the mucinase having a substantially similar mucin-hydrolyzing activity. A mucinase of the invention is particularly suitable for degrading mucus. The mucinase is particularly suitable of degrading mucus in a mammal, because a mammal naturally comprises a mucinase of the invention, which is endogenously present in the mammal. Therefore, a mucinase of the invention does not provoke severe side-effects and harmful immune responses in the mammal.

[0027] **Table I.** Human AMCase amino acid sequence (SEQ ID NO:1) deduced from cDNA sequence. The characteristic hydrophobic signal peptide (amino acids 1-21) is underlined with a single line.

MTKLILLTGLVLILNLQLGSAYQLTCYFTNWAQYRPGLG
RFMPDNIDPCLCTHLIYAFAGRQNEITTIEWNDVTLYQ
AFNGLKNKNSQLKTLLAIGGWNFGTAPFTAMVSTPENR
QTFITSVIKFLROYEFDGLDFDWEYPGSRGSPPODKHLFT
VLVQEMREAFEQEAKQINKPRLMVTAAVAAGISNIQSG
YEIPQLSQYLDYIHVMTYDLHGSGWEGYTGENSPLYKYP
TDTGSNAYLNV DYVMNYWKDNGAPAEKLIVGFPTYGH
NFILSNPSNTGIGAPTSAGAPYAKESGIWAYYEICTF
LKNGATQGWDAPOEVPYAYQGNVWVG YDNIKSFDIKA
QWLKHNKFGGAMVWAIDLDDFTGTFCNOGKFPLISTLK
KALGLQSASCTAPAQPIEPITAAPSGSGNGSGSSSSGGSS
GGSGFCAVRANGLYPVANNR NAFWHCVNGVTYQONC
QAGLVFDTSCDCCNWA

[0028] With a mucinase of the invention, it is now, for instance, possible to efficiently decrease an unwanted mucus barrier in the respiratory tract and/or gastrointestinal tract of a patient suffering from a disease in which mucus is involved without harmful side-effects. On the one hand, degrading mucus improves the capability of the patient to take up oxygen and/or nutrients. On the other hand, by (partially) degrading a thick mucus layer, delivery of other pharmaceutical compositions becomes easier, and mucociliary clearance improves, resulting in fewer persistent infections. It has also become possible to selectively counteract tumor cells which have a different mucus expression pattern as compared to normal cells. Additionally, it is possible to at least in part degrade microorganisms comprising mucus.

[0029] The invention also provides a mucinase produced by a host or host cell and isolated from the host or host cell or medium in which the host cell is cultured. In one embodiment, the amino acid sequence of the mucinase is encoded by a nucleotide sequence essentially corresponding to the nucleotide sequence shown in FIG-8 Table II below (SEQ

ID NO:2). The mucinase is called AMCase. Preferably, the mucinase has a molecular weight of about 50 kDa.

[0030] Table II. Human AMCase cDNA sequence (SEQ ID NO:2) (GenBank Accession Number AF290004).

gctttccagctcgtggtggaatcctccatagctgaagcctttgtgataaccacagaatcag
aacatataaaaagctctgcgggactggtgctgactgcaacatgacaaagcttattctcct
cacaggtcttgccttatactgaattgcagctcggtctgcctaccagctgacatgctact
caccaactgggccagctaccggccaggcctggggcgcttcatgcctgacaacatcgac
cctgcctctgtaccacctgatctacgccttgcctgggaggcagaacaacgagatcacc
accatcgaatggaacgatgtgactctctaccaagccttcaatggcctgaaaaataagaaca
gccagctgaaaactctcctggccattggaggctggaacttcgggactgccctttcactg
ccatggtttctactcctgagaaccgccagactttcatcacctcagtcataaattcctgcgc
cagtatgagttgacgggctggactttgactgggagtaccctggctcctggtggagccctc
ctcaggacaagcatctcttactgctcgtggtgcaggaaatgcgtgaagcttttgagcagga
ggccaagcagatcaacaagcccaggctgatggtcactgctgcagtagctgctggcatct
ccaataccagctcgtgatgatgagatcccccaactgtcacagtacctggactacatccatgtc
atgacctacgacctccatggctcctgggagggtactactggagagaaacagccccctcta
caaatacccgactgacaccggcagcaacgcctacctcaatgtggattatgtcatgaacta
ctggaaggacaatggagcaccagctgagaagctcatcgttgattccctacctatggaca
caacttcacctgagcaacccctcaacactggaattggtgccccacctctggtgctggt
cctgctgggccctatgccaaggagctctggatctgggcttactacgagatctgtaccttc
tgaanaatggagccactcagggatgggatgccctcaggaaatgccttatgcctatcag
ggcaatgtgtgggttgctatgacaacatcaagagcttcgatattaaggctcaatggctta
agcacaacaaattggaggcgccatggtctgggccattgatctggatgacttcactggca
ctttctgaaccaggggcaagtttccctaatactccacctgaagaaggccctcggcctgca
gagtgcgaagtgcacggctccagctcagccattgagccaataactgctgctcccagtgg
cagcgggaacgggagcgggagtagcagctctggaggcagctcgggaggcagtgat
tctgtgctgcagagccaacggcctctacccctggcgaataacagaaatgcctttggc
actgcgtgaatggagtcacgtaccagcagaactgccaggccgggctgtcttcgacacc
agctgtgattgctgcaactgggcataaacctgacctggtctatattccctagagtccagctc
cttttgcttaggacatgttcccctacctaagtcctgcaataaaatcagcagtc

[0031] By “a mucinase” is meant herein a proteinaceous molecule which is capable of, at least in part, hydrolyzing a mucin. This results in the cleavage of at least one sugar moiety bond of the mucin. Preferably, a sugar moiety, such as a β .1-4 linked N-acetylglucosamine, is cleaved. In terms of the invention, by “substantially isolated or purified” is meant that the mucinase is removed from an environment in which it naturally occurs, or that a sample, comprising the mucinase, is enriched for the mucinase. The sample may be obtained from a mammal, for instance, from a mouse or a human individual, because mammals endogenously comprise a mucinase of the invention.

[0032] By "a recombinant mucinase" is meant a mucinase which has artificially been made, as opposed to mucinases which are naturally generated in living organisms. A recombinant mucinase can, for instance, be generated by expression, either *in vitro* or *in vivo*, of a vector comprising a nucleic acid sequence encoding the mucinase.

[0033] In terms of the invention, "a sequence essentially corresponding to" means that variations of the sequence are allowed, as long as the variations do not alter the properties of the sequence in kind. The properties may, however, be somewhat altered in amount. For amino acid sequences, the variations, for instance, include a conservative substitution: a substitution of an amino acid residue with another amino acid residue with generally similar properties (such as size and hydrophobicity) such that the functioning of the amino acid sequence remains the same in kind, not necessarily in amount. Additionally, an amino acid residue may be deleted without significantly altering the function of the amino acid sequence. Generally, the sequence variations will be limited to less than 35%, preferably less than 20%, more preferably less than 10%. Therefore, the variants will generally have a homology of 65%, preferably 80 %, more preferably 90%. An amino acid sequence essentially corresponding to a mucinase, for instance, has the same kind of mucin-hydrolyzing property as the mucinase, though not necessarily in amount. Likewise, a nucleotide sequence essentially corresponding to a nucleotide sequence shown in ~~FIG. 8~~ Table II (SEQ ID NO:2) has the same kind of properties as that of the nucleotide sequence shown in ~~FIG. 8~~ Table II (SEQ ID NO:2). It encodes, for instance, a mucinase.

[0034] By "a modified form of the mucinase having a substantially similar mucine-hydrolyzing activity" is meant a molecule having substantially similar mucine-hydrolyzing activity, although the modified form may differ significantly from the mucinase. The modified form may, for instance, comprise a functional part of the mucinase. In terms of the invention, a functional part of a mucinase is defined as a part which has a substantially similar mucin-hydrolyzing activity as the mucinase. The functional part could, for instance, consist of the catalytic domain of the mucinase. The modified form may also be a functional derivative, wherein several domains are deleted and/or substituted and/or added. For instance, the modified form may comprise a fusion protein. The fusion protein, which is also herewith provided, preferably comprises a mucinase of the invention and/or a functional part thereof, and a protection moiety. The protection moiety allows for a longer half-life as compared to an unprotected mucinase of the invention and/or an unprotected functional part thereof. The protection moiety, for instance, comprises at least part of an immunoglobulin chain,

preferably a constant region of the chain. In one embodiment of the invention, a fusion protein of the invention comprises a human mucinase of the invention and/or a functional part thereof. A fusion protein of the invention preferably retains mucinase biological activity, both *in vitro* and *in vivo*, and preferably has an improved pharmacokinetics when administered *in vivo* as compared to an unprotected mucinase of the invention and/or an unprotected functional part thereof. The fusion protein can also comprise several copies of a desirable domain of the mucinase, and/or an additional domain which is not derived from the mucinase.

[0035] By "a substantially similar mucin-hydrolyzing activity" is meant herein the same mucin-hydrolyzing activity in kind, not necessarily in amount. Like mucinase, a compound with a substantially similar mucin-hydrolyzing activity is capable of cleaving at least one sugar moiety bond of mucin. The substantially similar mucin-hydrolyzing activity does not necessarily comprise additional (enzymatic) activities against other kinds of compounds. If, for instance, a mucinase of the invention comprises other catalytic activities besides its mucin-cleavage activity, a compound comprising a substantially similar mucin-hydrolyzing activity does not necessarily comprise the other catalytic activities.

[0036] In one aspect, the invention provides a pharmaceutical composition comprising an effective amount of a mucinase of the invention and a pharmaceutically acceptable carrier or diluent. The pharmaceutical composition is particularly suitable for therapeutic or prophylactic treatment of an individual against a disease in which mucus is involved, such as, for instance, cystic fibrosis, COPD, asthma, bronchitis, tuberculosis, a mucin-producing tumor and/or infection by a protozoan parasite. Preferably, the pharmaceutical composition further comprises a therapeutically or prophylactically effective amount of a second pharmaceutical composition, such as human DNase1, a mucolytic (*e.g.*, n-acetylcysteine), an antibiotic (*e.g.*, Tobramycin), a pancreatic enzyme supplement, an antifungal drug (*e.g.*, itraconazole, caspofungin), an antihistamine, a bronchodilator, a leukotrien inhibitor, and/or a corticosteroid.

[0037] In terms of the invention, a disease in which mucus is involved means that the disease is either associated with an altered mucin expression pattern in a patient or associated with a microorganism comprising mucus such as a protozoan parasite. The altered mucin expression pattern may lead to a thick mucus layer in the respiratory tract and/or gastrointestinal tract hampering the uptake of oxygen and/or nutrients and facilitating infections. Alternatively, the altered mucin expression pattern may only be induced locally.

This is, for instance, the case with carcinoma cells having an altered expression pattern as compared to normal cells.

[0038] The invention further comprises a composition comprising a mucinase of the invention and a carrier or diluent. For instance, the composition can be a medium for culturing cells, or a cosmetic, dental or food product.

[0039] Furthermore, the invention provides a method of therapeutic or prophylactic treatment of an individual against a disease in which mucus is involved, such as cystic fibrosis, COPD, asthma, bronchitis, tuberculosis, a mucin-producing tumor and/or infection by a protozoan parasite, comprising administering to the individual a pharmaceutical composition of the invention. The mucinase present in a pharmaceutical composition of the invention is capable of cleaving mucins. Therefore, with a method of the invention, it has, for instance, become possible to decrease an unwanted mucus barrier in the respiratory tract and/or gastrointestinal tract of a patient suffering or at risk of suffering from a disease in which mucus is involved. It has also become possible to specifically bind and/or cleave mucus of tumor cells having an altered mucus expression pattern. Once bound, the tumor cell can be subject to additional treatment by conventional pharmaceuticals. As another example, it has now also become possible to protect and/or treat an individual against a mucus-comprising pathogen, by prophylaxis and/or treatment according to a method of the invention

[0040] The invention also provides a process for preparing a mucinase of the invention, or a modified form thereof having a substantially similar mucin-hydrolyzing activity, comprising growing a host or a host cell capable of producing the mucinase or modified form thereof and isolating the mucinase produced from the host or host cell or from medium in which the host cell is cultured. In one aspect of the invention, the host or host cell is genetically engineered. Preferably, the amino acid sequence of the mucinase is encoded by a nucleotide sequence essentially corresponding to the nucleotide sequence shown in ~~FIG. 8~~ Table II (SEQ ID NO:2).

[0041] In another aspect of the invention, a mucinase of the invention is provided which further comprises a chitin-hydrolyzing activity. By "a chitin-hydrolyzing activity" is meant herein a capability of cleaving at least one bond of chitin. The mucinase is very suitable for degrading chitin, for instance, chitin from pathogenic microorganisms. Therefore, the invention also provides a pharmaceutical composition for therapeutic or prophylactic treatment of an individual against infection by a chitin-containing pathogen, comprising a

therapeutically or prophylactically effective amount of a mucinase of the invention and a pharmaceutically acceptable carrier or diluent. The invention also provides a method of therapeutic or prophylactic treatment of an individual against infection by a chitin-containing pathogen, comprising administering to the individual the pharmaceutical composition. The method is, for instance, very suitable for treating a CF-patient comprising *Aspergillus* species in its respiratory tract. With a method of the invention, a thick mucus layer and *Aspergillus* species can be degraded in the respiratory tract simultaneously, because of both mucus-hydrolyzing as well as chitin-hydrolyzing activity of the pharmaceutical composition. The method is also suitable for treatment of other pathogens in a mucosal lining, like, for instance, vulvovaginitis and ringworm.

[0042] A composition comprising a mucinase of the invention and a carrier or diluent is also herewith provided. For instance, the composition may be a medium for culturing cells, in particular human cells, or a cosmetic, dental, or food product. Furthermore, the invention provides a chitin-based article of manufacture comprising a chitin-hydrolyzing amount of a mucinase of the invention. The chitin-based article of manufacture may be a drug-containing carrier, an implant for controlled drug release or a transient functional implant.

[0043] An isolated host cell capable of producing a mammalian mucinase of the invention is also herewith provided, as well as a recombinant nucleic acid comprising a nucleotide sequence encoding, or complementary to a nucleotide sequence encoding, an expressible mammalian mucinase of the invention. The mucinase may comprise an amino acid sequence essentially corresponding to the amino acid sequence shown in ~~FIG. 8~~ Table I (SEQ ID NO:1). Preferably, the nucleotide sequence essentially corresponds to, or essentially is complementary to, the nucleic acid sequence shown in ~~FIG. 8~~ Table II (SEQ ID NO:2). By "a nucleotide sequence encoding an expressible mucinase" is meant herein a nucleotide sequence encoding a mucinase that at least in part can be obtained by transcription and/or translation of the nucleotide sequence. By "essentially complementary to a nucleic acid sequence" is meant that a particular nucleic acid can bind by hybridization to the nucleic acid sequence, especially under stringent conditions. By "essentially corresponds to the nucleic acid sequence shown in ~~FIG. 8~~ Table II" (SEQ ID NO:2)" is meant herein that a nucleotide sequence codes for the same amino acid sequence that is encoded by the nucleic acid sequence shown in ~~FIG. 8~~ Table II (SEQ ID NO:2), and/or codes for a modified form of the amino acid sequence having a substantially similar mucin-hydrolyzing activity. A

nucleotide sequence coding for the same amino acid sequence can, for instance, utilize a different codon usage.

[0044] Also provided herewith is an oligonucleotide of at least about 8 nucleotides having a nucleotide sequence corresponding to, or complementary to, a nucleotide sequence shown in ~~FIG. 8~~ Table II (SEQ ID NO:2) and being capable of binding by hybridization under stringent hybridization conditions to nucleic acid coding for a mucinase of the invention. The oligonucleotide is useful for different purposes. For instance, the oligonucleotide can be used as a probe in a hybridization analysis, or as a primer in a nucleic acid amplification method such as PCR, NASBA, etc. The invention also provides a peptide of at least about 8 amino acid residues having an amino acid sequence derived from the amino acid sequence shown in ~~FIG. 8~~ Table I (SEQ ID NO:1) and representing or mimicking an epitope of a mucinase of the invention, in particular those having an amino acid sequence corresponding to an amino acid sequence shown in ~~FIG. 8~~ Table I (SEQ ID NO:1) and having antigenicity. Usually, such peptides will have a length of at least about 10, or even at least about 15, or at least about 40 amino acid residues. Preferably, the peptide comprises a length of about 30 amino acid residues. The peptides are, for instance, suitable for diagnostic purposes, or in immunization protocols to raise mammalian mucinase-specific antibodies.

[0045] An antibody capable of binding to a mucinase of the invention is also herewith provided. Preferably, the antibody is a monoclonal antibody. An antibody of the invention can be used for many purposes, for instance, for isolating and/or purifying (*e.g.*, by affinity chromatography) a mucinase of the invention.

[0046] In yet another aspect, the invention provides a diagnostic kit comprising an antibody of the invention, and/or a peptide of the invention, and/or a diagnostically effective amount of a mucinase of the invention, and a conventional component of diagnostic kits for detecting an antigen or an antibody. Also provided is a diagnostic kit comprising an oligonucleotide of the invention and/or a recombinant nucleic acid of the invention, and a conventional component of diagnostic kits for detecting a nucleic acid.

[0047] Furthermore, the invention provides a method of decomposing mucin comprising contacting the mucin with a mucinase of the invention under mucin-hydrolyzing conditions. The invention also provides a method of decomposing chitin comprising contacting the chitin with a mucinase of the invention which further comprises a chitin-hydrolyzing activity, under chitin-hydrolyzing conditions.

LEGENDS OF THE DRAWINGS

[0048] FIG. 1. Isoelectric focusing profile of chitinolytic activity in mouse lung extract. Isoelectric focusing was performed as described in experimental procedures. Chitinolytic activity was measured using 4MU-chitotrioside substrate. The enzyme activity present in the different isoelectric focusing fractions is expressed as a percentage of the total activity present in all fractions.

~~[0049] FIG. 2. Mouse AMCase cDNA sequence and deduced amino acid sequence (SEQ ID NO:). The cDNA sequence (GenBank Accession Number AF290003) is indicated by the upper sequence and the deduced amino acid sequence is depicted below the nucleotide sequence. The characteristic hydrophobic signal peptide (amino acids 1-21) is underlined with a single line. The putative chitin binding domain (amino acids 426-473) is underlined with a double line. The hinge region separating the catalytic domain from the chitin binding domain is underlined with a dashed line. The part of the protein purified from mouse intestine that was determined by Edman sequencing is boxed.~~

[0050] FIG. 32. Degradation products with colloidal chitin as substrate. The FACE technique (described in experimental procedures) was used to visualize the cleavage products of recombinant human chitotriosidase and recombinant mouse AMCase using colloidal chitin as substrate. Lane 1, no enzyme added. Lane 2, products formed after incubation with 50 kDa recombinant human chitotriosidase with chitin. Lane 3, products formed with recombinant mouse AMCase and chitin. Lane 4, human chitotriosidase incubated without substrate. Lane 5, mouse AMCase incubated without substrate. Marker lane is indicated with M (sugar polymers are indicated on the right-hand side).

[0051] FIG. 43. Electrophoretic behavior of chitinases.

Panel A: Purified recombinant human chitotriosidase and mouse AMCase were separated on a 12.5% SDS-PAGE gel in the presence or absence of a reducing agent and visualized by silver staining as described in experimental procedures (panel A). Lane 1, recombinant mouse AMCase under reducing conditions. Lane 2, recombinant human chitotriosidase under reducing conditions. Lane 3, recombinant human chitotriosidase under non-reducing conditions. Lane 4, recombinant mouse AMCase under non-reducing conditions. M indicates the molecular weight standards (mass (kDa) indicated at the left-hand side).

Panel B: The same purified recombinant enzymes as described in panel A were separated on a 10% SDS-PAGE gel containing glycol-chitin as described in experimental procedures. Chitinolytic activity was visualized as clearing zones in the gel. Lane 1, recombinant human

39 kDa chitotriosidase. Lane 2, recombinant human 50 kDa chitotriosidase. Lane 3, recombinant mouse AMCase (mass (kDa) indicated at the right-hand side).

[0052] FIG. 54. Effects of acidic pH.

Panel A: pH activity profile of the different chitinases. The pH optima were determined by monitoring enzyme activity at the indicated pH in McIlvaine buffer. Purified human recombinant chitotriosidase (closed lozenge), purified mouse AMCase (closed circle).

Panel B: Effects of acidic pre-incubation. Purified recombinant human chitotriosidase and mouse AMCase were pre-incubated for 30 minutes at the indicated pH in McIlvaine buffer prior to enzyme activity measurement at the assay pH (see experimental procedures). Activity prior to incubation at the indicated pH is defined as 100%.

Panel C: Precipitation by trichloroacetic acid (TCA). Purified recombinant human chitotriosidase and mouse AMCase were incubated with the indicated percentages of TCA. The amount of remaining enzyme activity after centrifugation is shown as a percentage of initial amounts.

[0053] FIG. 65. Tissue distribution of mouse AMCase mRNA.

Panel A: The relative expression levels of mouse AMCase in various mouse tissues as determined by dot blot analysis using an RNA Master Blot (Clontech) as described in experimental procedures. The highest level of expression is defined as 100%.

Panel B: Northern blot of RNA isolated from the indicated mouse tissues. 15 micrograms total of RNA was separated on an agarose gel as described in experimental procedures. The full-length mouse AMCase cDNA was used as probe. As a control for RNA loading, a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was used (data not shown). The position of the 18S ribosomal RNA band is indicated.

[0054] FIG. 76. Tissue distribution of human AMCase mRNA. The relative expression levels of human AMCase in various human tissues were determined by dot blot analysis using an RNA Master Blot (Clontech) using the oq35c04,s1 EST clone (GenBank Accession Number AA976830) as probe. The highest level of expression is defined as 100%. Several tissues were excluded from the figure since they did not result in a detectable signal: amygdala, caudate nucleus, cerebellum, cerebral cortex, frontal lobe, hippocampus, medulla oblongata, occipital lobe, putamen, substantia nigra, temporal lobe, thalamus, nucleus accumbens, spinal cord, fetal brain, fetal heart, fetal kidney, fetal liver, fetal spleen and fetal thymus.

[0055] ~~FIG. 8 (SEQ ID NO: __). Human AMCase cDNA sequence and deduced amino acid sequence.~~

~~Panel A: The human AMCase cDNA sequence (GenBank Accession Number AF290004) is indicated by the upper sequence and the deduced amino acid sequence is indicated below the nucleotide sequence. The characteristic hydrophobic signal peptide (amino acids 1-21) is underlined with a single line.~~

~~Panel B: Amino acid sequence comparison of mature (without signal peptide) human (h) and mouse (m) AMCase and human chitotriosidase. Residues conserved among at least two out of the three sequences are boxed.~~

[0056] FIG. 97. The effect of AMCase on glycoproteins was studied by evaluating the effect of mouse AMCase on mucin, a type of glycoproteins expressed on mucosal surfaces in the airways and gastrointestinal tract. Bovine submaxillary gland mucin (Sigma) 125 µg was dissolved in 40 µl 0.05M NaAc pH 5.0 in the presence or absence of 500 ng mouse AMCase. After overnight incubation at 37°C, the mucin was electrophorized on an SDS-PAGE gel 7.5%, followed by silver staining. As is shown in FIG. 97, the mucin was degraded substantially by treatment with the AMCase (left lane), but not when it was treated with control buffer (right lane).

[0057] FIG. 8. Amino acid sequence comparison of mature (without signal peptide) human (h) (SEQ ID NO:14) and mouse (m) (SEQ ID NO:9) AMCase and human chitotriosidase (SEQ ID NO:10). Residues conserved among at least two out of the three sequences are in bold.

DETAILED DESCRIPTION OF THE INVENTION

[0058] Mucus provides an important defensive barrier which forms the first line of defense to the external environment. However, several diseases involve a disturbed generation of mucus, resulting in thick and adhesive mucus. This leads, among others, to problems according to the uptake of oxygen and nutrients by a patient. Additionally, because of the thick adhesive mucus, current pharmaceutical compounds have a reduced capability to reach their targets (for instance, epithelial cells or DNA present in the mucus), and opportunistic infections occur as a result of impaired mucociliary clearance.

[0059] Furthermore, infections with mucus-containing pathogens like protozoan parasites can induce severe complications. The same applies to several other pathogens present in mucosal linings of an individual, for instance, the fungus *Aspergillus*, in the lungs.

[0060] Although pharmaceutical compositions are currently used to counteract diseases in which mucus is involved, there is yet no efficient and satisfactory method to degrade unwanted mucus.

[0061] For cystic fibrosis and COPD, the most widely used mucolytic agents are n-acetylcysteine or acetylcysteine and recombinant human DNase. Other mucolytics used in clinic include guaifenesin, carbocysteine lysine, citiolone, sobrerol, ambroxol, myrtol, iodinated glycerol, isobutyrylcysteine, and letosteine.

[0062] N-acetylcysteine is an aerosolized mucolytic agent often used as adjunctive therapy for pulmonary complications of cystic fibrosis. The viscosity of mucous secretions in the lungs is dependent upon the concentrations of mucin and DNA. N-acetylcysteine acts to split the sulfide bonds between DNA and mucins, thereby decreasing mucus viscosity. The action of N-acetylcysteine is pH dependent. Mucolytic action is significant at ranges of pH 7.9 (Kastrup et al., 1998).

[0063] Adverse effects reported with acetylcysteine include stomatitis, nausea, vomiting, hemoptysis, and severe rhinorrhea. Acetylcysteine has an unpleasant, pungent odor that may lead to an increased incidence of nausea. Bronchoconstriction has also been reported with acetylcysteine therapy. N-acetylcysteine has mucolytic activity *in vitro*; this activity has, however, not been demonstrated convincingly *in vivo* (Celli et al., 1995). A possible explanation for this finding is a pH in the airways that is lower than 7.0. Even though the normal tracheal mucus pH ranges from 6.9-9.0, in infection, pH values can become as low as pH 5.8 in mucus (Buhrmester 1933). An acidic pulmonary environment has been reported for at least two mucus-associated lung diseases, namely asthma (Hunt et al.) and cystic fibrosis (Choi et al.). A similar decreased pH may also be present in the lungs of COPD patients, as they have respiratory acidosis (Plant et al.).

[0064] DNA is a factor that contributes to viscous mucus in CF patients. This high extracellular DNA (as high as 3-14 mg/ml, Chernick and Barbero 1959, Potter et al., 1960) content further thickens airway secretions. Recombinant human DNase (Pulmozyme) has been demonstrated to reduce the viscosity of sputum in CF patients by hydrolyzing the extracellular DNA (Shak et al., 1990, Zahm et al., 1995). DNase is a highly purified solution of recombinant human deoxyribonuclease I (rhDNase), an enzyme that selectively cleaves

DNA. Studies have demonstrated that daily administration of recombinant human DNase resulted in definite improvement in pulmonary function, as assessed by FEV1, above baseline (Fuchs et al., 1994, Shak et al., 1995). Recombinant human DNase is indicated in the management of patients with CF to improve pulmonary function and decrease the frequency of respiratory infections. Safety and efficacy have not been demonstrated in children less than 5 years of age. The recommended dose of recombinant human DNase for most patients with CF is 2.5 mg by nebulization once daily. Adverse effects include voice alteration, pharyngitis, laryngitis, rash, and chest pain. As DNase only targets the DNA present in viscous sputum, the mode of action of AMCase is independent of DNase, and possibly synergistic. In addition, for indications as COPD, where less DNA is present in the sputum, AMCase is more effective.

[0065] Although pharmaceutical compositions are currently used to counteract diseases in which mucus is involved, it has to be concluded that there is an unmet need for more sophisticated agents to degrade thick, adhesive mucus and to treat and/or prevent chronic infections with pathogens in epidermal and mucosal body linings. Preferably, such agents should be highly specific and effective and should not be prone for developing resistance there against and neither cause toxic side effects.

[0066] It was previously disclosed that bacterial chitinase preparations, which are expected to be highly immunogenic when administered to humans, can degrade human ocular mucus (Argueso et al., 1998). Here it is disclosed that mammals naturally comprise a mucinase. The mammalian mucinase, endogenously present at mucosal surfaces in mammals, provides the solution for the above-mentioned problems concerning diseases in which mucus is involved. A mucinase of the invention is capable of cleaving mucus. In one embodiment, a specific mouse (nucleotide sequence SEQ ID NO:3 (see, Table III, below) and amino acid sequence SEQ ID NO:4 (see, Table IV, below) and human mucinase (nucleotide sequence SEQ ID NO:2 (see, Table II, above) and amino acid sequence SEQ ID NO:1 (see, Table I, above)) is provided, ~~called AMCase, which is depicted in more detail in FIGS. 2 and 8 (SEQ ID NO:—).~~ It has a catalytically active 39 kDa domain which is connected via a hinge region with a C-terminal mucin-binding domain. To show its mucus-degrading property, we have incubated a mixture of submaxillary gland mucins with recombinant 50 kDa AMCase. The effect of the incubation was examined by analysis with SDS-PAGE and silver staining. FIG. 97 shows that a remarkable reduction in size of the mucins occurs. It was noted that the viscosity of the mucin solution was markedly reduced following incubation

with AMCase. The flow rate of the mucin solution, as measured in a vertically positioned glass pipette, was increased with almost a factor of 2.

[0066] **Table III.** Mouse AMCase cDNA sequence (SEQ ID NO:3) (GenBank Accession Number AF290003).

atggccaagctacttctcgtcacaggctcggctcttctgctgaatgctcagctggggtctgc
ctacaatctgatatgctatttcaccaactgggccagtatcgccaggtctgggagcttc
aagcctgatgacattaacccctgcctgtgtactcacctgatctatgccttgcctgggatgca
gaacaatgagatcaccaccatagaatggaatgatgttactctctataaagcttcaatgactt
gaaaaacaggaacagcaaaactgaaaaccctcctggcaattggaggctggaacttggaa
ctgctccttctactaccatggttccacttctcagaaccgccagaccttattacctcagtcac
caaatttctgcgtcagtatgggttgatggactggacctggactgggaataccagggtca
cgtgggagccctcctcaggacaagcatcttctactgtcctgtaaggaaatgcgtgaag
ctttgagcaggaggctattgagagcaacaggcccagactgatgttactgctgctgtag
ctgggtggatttccaacatccaggctggctatgagatccctgaacttctaagtacctggat
ttcatccatgtcatgacatatgacctccatggctcctgggagggtctacactggggagaata
gtcctctttacaataacccactgagactggtagcaatgcctacctcaatgtggattatgca
tgaactattggaagaacaatggagccccagctgagaagctcattgttgattcccagagt
atggacacaccttcacctcctgagaaacccctctgataatggaattggtccccctacctcgtg
gatggccctgctggcgcctataccagacaggctgggttctgggcctactatgagattgc
acctttctgagaagtggagccactgaggtctgggatgctcccaagaagtgccctatgcc
tataaggccaacgagtggcttggctatgacaatacaagagcttcagtgtaaggctcagt
ggcttaagcagaacaatttggaggtgccatgatctgggccattgaccttgatgacttact
ggctctttctgtgatcagggaatttctctgacttctactttgaacaaagcccttggcatat
ccactgaagggtgcacagctcctgacgtgccttccgagccagtgactactcctccaggaa
gtgggagtgggggtggaagctccggagggaagctctggaggcagtggtattctgtgccga
caaagcagatggcctctaccctgtggcagatgacagaaatgcttttggcagtgcatcaat
ggaatcacataccagcagcattgtcaagcagggtgttttgataccagctgtaattgtg
caactggccatgaacctaattgccattctccagaaatttctgcactctccttactcctacca
aaagtaactatctcccttaaccttatgcaataaaattgtagccaaaaca

[0067] **Table IV.** Mouse AMCase amino acid sequence (SEQ ID NO:4) deduced from cDNA sequence (SEQ ID NO:3). The characteristic hydrophobic signal peptide (amino acids 1-21) is underlined with a single line. The putative chitin-binding domain (amino acids 426-473) is underlined with a double line. The hinge region separating the catalytic domain from the chitin-binding domain is underlined with a dashed line. The part of the protein purified from mouse intestine that was determined by Edman sequencing is italicized.

MAKLLLVTGLALLLNAQLGSAYNLCYFTNWAQYRPLG
SFKPDDINPCLCTHLIYAFAGMONEITTIEWNDVTLYK
AFNDLKNRNSKLLKTLAIGGWNFGTAPFTTMVSTSONR
QTFITSVIKFLROYGFDGLDLWEYPGSRGSPQDKHLF
TVLVKEMREAFEQEAIESNRPRMLMVTA AVAGGISNIOAG
YEIPELSKYLDFIHVMTYDLHGSWEGYTGENSPLYKYPT
ETGSNAYLNVDYVMNYWKNNGAPAEKLIVGFPEYGH
FILRNPSDNGIGAPTS GDGPAGAYTROAGFWAYYEICTF
LRSGATEVWDASQEVPIYAYKANEWLGYDNIKSFSVKA

OWLKONNFGGAMIWAIDLDDFTGSFCDQGKFPLTSTLN
KALGISTEGCTAPDVPSEPVTTPPGSGSGGGSSGGSSGGS
GFCADKADGLYPVADDRNAFWOCINGITYOOHCOAGL
VFDTSCNCCNWP

[0068] A thick and adhesive mucus layer in the respiratory tract and/or gastrointestinal tract of an individual with, for instance, CF, COPD, or asthma can be, at least in part, degraded by administration of a mucinase of the invention to the respiratory tract and/or gastrointestinal tract of an individual. (Partly) degradation of the thick mucus layer enhances oxygen and/or nutrient uptake by the individual. Additionally, it enhances the capability of other medicaments to reach their target and prevents the occurrence of persistent chronic infections. Because a mucinase of the invention is endogenously present in mammals, it does not provoke severe immunological reactions after additional administration of the mucinase to the mammals.

[0069] A pharmaceutical composition or commercial formulation typically comprises a certain percentage of Active Pharmaceutical Ingredient, i.e., purest achievable form of a mucinase of the invention, as well as a suitable carrier that ensures the composition to be an appropriate delivery vehicle to the area of disease. Examples are a cream or ointment for skin infections, tablet or capsule formulations for the digestive tract, and an inhalation formulation for the pulmonary airways. Appropriate doses will be formulated such that these are the Maximum Effective Dose with the appropriate safety and tolerability profile.

[0070] In order to use a mammalian mucinase, like AMCase, as a pharmaceutical agent against thick mucus layers, the mammal has to be tolerant for the mucinase. Because a mucinase of the invention occurs naturally in the mammalian body, no strong immune response is elicited by additional administration of the enzyme to the mammal.

[0071] Another requirement in the application of an enzyme as a therapeutic agent is its ability to survive and to be functional in the body. This requirement is met because a mucinase of the invention is a remarkably stable enzyme. For instance, mouse AMCase can endure incubation at acidic (pH 2) up to quite basic conditions (pH 8). The enzyme is quite resistant against various proteases.

[0072] A mucinase of the invention preferably has a low pH optimum. For instance, mouse AMCase shows a pronounced pH optimum at pH 2.3 and a less pronounced optimum at pH 4.7. A mucinase of the invention with a low pH optimum is very suitable, especially because it is pH stable, for enhancing food digestion and normal bowel movement in patients with a thick mucus layer in the gastrointestinal tract, like, for instance, cystic

fibrosis patients. It can, for instance, well perform its catalytic action in the acidic stomach, whereas many other pharmaceutical compounds are inactivated.

[0073] A mucinase of the invention can be combined with existing medicaments. Once (part of) a thick mucus layer is cleaved and/or bound by mucinase, other pharmaceuticals are more capable of reaching their targets. In one embodiment, the invention therefore provides a mucinase conjugate comprising a mucinase of the invention and a second molecule, like, for instance, a second pharmaceutical compound and/or an immunoglobulin chain. The mucinase is preferably bound to the second molecule. The conjugate is suitable for a combined therapy. For instance, the conjugate can cleave mucus in the lungs and/or gastrointestinal tract of a patient with its mucin-hydrolyzing catalytic domain, after which the second pharmaceutical compound is capable of performing its therapeutic task. Alternatively, the conjugate can bind mucus with its mucus-binding domain without cleaving the mucus, after which the second pharmaceutical compound is capable of performing its therapeutic task.

[0074] Possible AMCase combination therapies are:

CF: DNase 1 (pulmozyme, Genentech) and AMCase

Oral AMCase and pancreatic enzyme supplements

AMCase and antibiotics

AMCase and gene therapy

CF with ABPA: itraconazole (or other antifungal treatments, for ABPA itraconazole is most often used) with AMCase (+ other CF medication) +/- oral corticosteroids

Asthma: AMCase with antihistamines, bronchodilators or corticosteroids.

Asthma with ABPA: itraconazole (or other antifungal treatments, for ABPA itraconazole is most often used) with AMCase +/- antihistamines, bronchodilators or corticosteroids

ABPA without asthma or CF: oral corticosteroids and AMCase (topical administration)

Chronic obstructive pulmonary disease with bronchitis: AMCase alone, or in combination with bronchodilators, b-adrenergic agents, methylxanthines, corticosteroids, or mucolytics, (mostly with n-acetylcysteine: flumocyl or mucomyst) is efficacious.

[0075] Systemic indications for a mucinase of the invention are also foreseen. These include but are not limited to mucus-producing benign and malignant tumors. Mucinase can be administered alone or in combination with other treatments.

[0076] A mammalian mucinase can additionally comprise chitin-hydrolyzing activity. For instance, we have found that mouse and human AMCase also comprise chitin-hydrolyzing activity. This is an important finding, because 6% of the human individuals are deficient for the only human chitin-hydrolyzing enzyme known, chitotriosidase. In those individuals, an endogenic mucinase can take over that function.

[0077] Next to cellulose, chitin is the most abundant glycopolymer on earth, being present as a structural component in coatings of many species, such as the cell wall of most fungi (Debono and Gordee 1994), the microfilarial sheath of parasitic nematodes (Fuhrman and Piessens 1985, Araujo et al., 1993), the exoskeleton of all types of arthropods (Neville et al., 1976) and in the lining of guts of many insects (Shahabuddin and Kaslow 1994). Chitinases (EC 3.2.1.14) are endo- β -1,4-N-acetylglucosaminidases that can fragment chitin and have been identified in several organisms (Flach et al., 1992). Until a few years ago it was generally assumed that man lacks the ability to produce a functional chitinase.

[0078] Chitin-containing pathogens like fungi constitute a common treat of infection of mammals. The mammalian immune system governs a broad array of defense mechanisms against systemic fungal infections. However, the incidence of life-threatening systemic fungal infections is rapidly increasing as the result of increasing active suppression of the immune system of patients during medical interventions, for example, during chemotherapies and transplantations, as well as due to viral suppression of the immune system, for example, during AIDS. Present antifungal agents show serious limitations such as the increasing resistance among the major human pathogenic fungi against existing drugs like azoles or because of the limited efficacy and toxic side effects of antifungal compounds like amphotericins. It is therefore to be concluded that there is an unmet need for more sophisticated agents to treat and/or prevent chronic infections with chitin-containing pathogens in epidermal and mucosal body linings. Preferentially, such agents should be highly specific and effective and should not be prone for developing resistance there against and neither cause toxic side effects.

[0079] The features of a mucinase of the invention which further comprises chitin-hydrolyzing activity (endogenous production in mucosal body linings, extreme acid pH stability and protease resistance, potent fungistatic action) make the mucinase an ideal candidate for use as a drug against topical/mucosal infections with chitin-containing pathogens such as fungi.

[0080] As chitin-containing pathogens such as fungi enter the body via accessible sites such as the nose, mouth, lungs, ears, eyes, skin, urethra, bladder, topical infections of these sites occur frequently. These sites are in contact with the outside world, and as a result, topical administration of antifungal drugs may be necessary to achieve optimal efficacy.

[0081] Prominent topical fungal infections for AMCase indication are, for instance, allergic bronchopulmonary aspergillosis (ABPA), vaginal infection with candida (vulvovaginitis: Low pH; a mucinase of the invention is active whereas chitotriosidase is not), dermatophytosis (ringworm of skin, scalp, nails and athlete's foot), and other topical fungal infections in body linings (including nonallergic lung aspergillosis).

[0082] Current treatment of fungal infections include allylamines (mostly topical applications) antimetabolites (oral, IV), azoles (topical, oral, IV), glucan synthesis inhibitors (IV), polyene macrolides (mostly IV, amphotericin B and derivatives, nystatin topical, pimarinic ophthalmic), and other drugs (systemic: Griseofulvin, topical: ciclopiroxolamine, haloprogin, tolnaftate, undecylenate). Many of these drugs, especially the ones for invasive and systemic infections, are hampered by serious side effects. Such side effects are less likely if a mucinase of the invention which further comprises chitin-hydrolyzing activity, which is endogenously expressed, is used as a protein drug. Thus, a mucinase of the invention which further comprises chitin-hydrolyzing activity is very suitable for treatment of lung infections in, for instance, cystic fibrosis and COPD.

[0083] Allergic bronchopulmonary aspergillosis (ABPA) is a syndrome seen in patients with severe obstructive lung disease, most commonly in asthma and cystic fibrosis (Cockrill & Hales 1999). Chronic colonization of the airways by *Aspergillus* is apparent in these patients, accompanied by *Aspergillus*-specific IgE antibody production and eosinophilia. ABPA is generally treated by using oral corticosteroids such as prednisone to suppress the inflammatory component of the disease. Antifungal therapy, such as treatment with the orally active itraconazole, has been reported. However, no controlled trials have been reported. In addition, as itraconazole is used for systemic treatment, *Aspergillus* in the airway lumen may not be treated as efficiently. Therefore, novel treatments need to be developed to treat fungal infections in these pulmonary diseases. A mucinase of the invention which further comprises chitin-hydrolyzing activity is suitable for use for this indication.

[0084] A mucinase of the invention which further comprises chitin-hydrolyzing activity is also suitable in the event of resistance to current antifungal therapies. For instance,

flucytosine resistance is common, especially when used as monotherapy for candida. Adverse effects are displayed as well.

[0085] Resistance to ketoconazole described with prolonged treatment of AIDS patients, adverse effects indications mucosal candidiasis, mycoses, histoplasmosis, dermatophytes. Resistance to fluconazole is increasing in AIDS patients, and adverse effects are displayed. The indications are mucosal candidiasis and vulvovaginal candidiasis. Resistance to itraconazole is not clear. There are few side effects. There is an indication of candida histoplasmosis, mycoses, aspergillosis, sporotrichosis, candida Terbinafine, especially to dermatophytes.

[0086] A mucinase of the invention which further comprises chitin-hydrolyzing activity can also be administered in combination with another current pharmaceutical. The invention therefore also provides a mucinase conjugate, comprising a mucinase of the invention which further comprises chitin-hydrolyzing activity and a second molecule, for instance, a second pharmaceutical compound and/or an immunoglobulin chain. Preferably, the mucinase is bound to the second molecule. The mucinase conjugate can bind chitin with its chitin-binding domain after which the second pharmaceutical compound is capable of performing its therapeutic task. The mucinase can also cleave the chitin with its chitin-hydrolyzing catalytic domain, but this is not necessary. A possible combination therapy for treatment of a fungus infection is: azoles, antimetabolites, glucan synthesis inhibitors, griseofulvin (all with intracellular activity) together with a mucinase of the invention which further comprises chitin-hydrolyzing activity, like, for instance, AMCase. Of course, the several pharmaceutical compositions do not necessarily have to be administered at the same time. They can be administered together or separately, with either the same or different administration doses and administration intervals.

[0087] In one aspect, the invention provides a mucinase of the invention which has a low pH optimum. A major advantage of a mucinase of the invention which has a low pH optimum is that the mucinase even withstands the harsh conditions in the gastrointestinal tract and can therefore be administered orally.

[0088] A mucinase of the invention which has a low pH optimum is also suitable for use as a topical agent. For instance, athlete's foot, a topical infection caused by trichophyton or epydermophyton, involves a local decrease of pH. Therefore, a mucinase of the invention which has a low pH optimum and which has a chitin-hydrolyzing activity is particularly suitable for topical treatment of athlete's foot and for topical treatment of any

pathogen involving a low pH, like, for instance, a vaginal infection by *Candida albicans*. For instance, incubation of hyphae of *Candida albicans* with recombinant AMCase results in selective lysis of the growing tip, similar to the effect of chitotriosidase.

[0089] Additional important advantages of a mucinase of the invention which further comprises chitin-hydrolyzing activity compared to classic antifungal agents are the following. In the first place, since a mucinase of the invention which further comprises chitin-hydrolyzing activity is an endogenous protein, its administration will not result in a severe immune reaction. In the second place, resistance of fungi against chitinases has not been developed so far and seems intrinsically difficult given the fact that, despite the evolutionary pressure, plant fungal pathogens have remained sensitive to chitinases.

[0090] To counteract chitin-containing pathogens, like fungi, a mucinase of the invention which further comprises chitin-hydrolyzing activity, like AMCase, can be administered locally as a crème, for instance, on the skin. It may also be used for treatment of local ear-infections or vagina-infections, such as vulvovaginitis. Especially the latter also involves an acidic environment, in which a mucinase of the invention which further comprises chitin-hydrolyzing activity and a low pH optimum, like AMCase, is very well capable to perform its catalytic actions.

[0091] Body locations of a mucinase of the invention have led us to the conclusion that the mucinase can perform additional functions next to mucus degradation and defense against chitin-containing pathogens. For instance, the remarkably high concentration of AMCase in the gastrointestinal tract involves a role in food processing during the evolution of mammals.

[0092] A chitin-hydrolyzing capability of a mammalian mucinase, for instance, AMCase, can also be exploited as a tool to degrade injected or implanted chitin-based structures for medical purposes. For instance, drugs can be incorporated in chitin-based capsules. The concomitant presence of well-defined amounts of a mammalian mucinase, which further comprises chitin-hydrolyzing activity, in the capsule ensures a controlled release of drugs. A slow but gradual release of drugs is particularly envisioned when the drugs are trapped in a chitin matrix. The use of a the mucinase in such a system results in ultimate destruction of the chitin-based capsule and does not elicit an immunological response. The drugs used in such a system can vary from small compounds to protein and DNA fragments for the purpose of enzyme and gene therapy. Chitin (or analogues thereof) is already employed as a carrier for drugs.

[0093] Another application is the use of a mammalian mucinase which further comprises chitin-hydrolyzing activity for the swift degradation of implants that contain chitin as a structural component. This is useful in the case of implants that only temporarily have to fulfill a function and can be conveniently degraded by administration of a mammalian mucinase which further comprises chitin-hydrolyzing activity, like AMCase.

[0094] A mucinase of the invention which further comprises chitin-hydrolyzing activity and a low pH optimum, like AMCase, is especially suitable for the above-mentioned applications in the case that an acid environment is involved, or additional mucus degradation is required.

[0095] A mammalian mucinase which further comprises chitin-hydrolyzing activity can also be used *ex vivo* for degradation of mucus-containing and/or chitin-containing microorganisms. For instance, as a preventive measure, a mucinase of the invention can be added to a culture medium of cells. The cells may preferably be cultured in the absence of antibiotics. Examples are the *ex vivo* culture of cells for the purpose of gene therapy and the *ex vivo* culture of keratinocytes to be used in connection with wound healing.

[0096] A mucinase of the invention is as well suitable as an additive in toothpaste and body lotions in order to prevent infections with mucus-containing and/or chitin-containing microorganisms. Additionally, a mucinase of the invention can be used as a food preservative. For instance, it can inhibit growth of mucus-containing and/or chitin-containing pathogens in food.

[0097] The N-terminal amino acid sequence of purified AMCase was determined (Boot et al., 2000) (~~see, FIG. 2~~see, Table III, above). The N-terminal amino acid sequence allowed the cloning of the corresponding full-length mouse AMCase cDNA, (Boot et al., 2000). The full-length cDNA predicts the synthesis of a 50 kDa (pI 4.85) protein with a characteristic signal peptide (~~see, FIG. 2~~see, Table IV, above). Expression of this cDNA in COS cells led to secretion of a 50 kDa active mucinase/chitinase with a pI of 4.8. AMCase was found to bind to chitin particles with high affinity. Chitin affinity chromatography was used to purify the enzyme, as described in experimental procedures. The procedure resulted in a 30.082-fold purification of an apparently homogeneous 50 kDa protein. The specific activity of the purified enzyme was 3.9 nmol 4-methylumbelliferyl-chitotrioside hydrolyzed at pH 5.2 per mg per hour, being almost identical to that of chitotriosidase. The catalytic domain of mouse AMCase is also herewith provided.

[0098] Mouse AMCase mRNA is predominantly found in stomach, submaxillary gland and also at a lower level in the lung (see, FIG. 65). Surprisingly, no mouse AMCase mRNA can be detected in the small intestine, suggesting that the protein in the intestine is probably derived from the upper parts of the gastrointestinal tract, such as the stomach.

[0099] Our findings demonstrate that AMCase in mammals is distinct from chitotriosidase: the newly discovered, discrete enzyme is referred to as acidic mammalian chitinase or AMCase. AMCase is also present in man. Screening the human EST database at the NCBI with the acidic mouse chitinase cDNA revealed the presence of a human EST clone (oq35c04.s1, Genbank acc. no. AA976830) that is highly homologous to the acidic mouse chitinase. The tissue distribution of the human mRNA was examined using a human Masterblot (Clontech). The expression pattern of this mRNA is similar to the expression pattern of the acidic mouse chitinase (FIG. 65), being highly expressed in the stomach and at a lower level in the lung. Using degenerate oligonucleotides directed against members of the chitinase family, we were able to amplify other regions of the human acidic chitinase, generating enough information to clone the full-length human acidic chitinase cDNA. Screening the Genbank database using the full-length human cDNA revealed that it was almost identical to TSA1902-L and TSA1902-S from a lung cDNA library described by Saito et al. (Saito et al., 1999). These two sequences are most probably splice variants of the acidic human chitinase mRNA. Only expression of full-length human AMCase cDNA in COS cells led to the production of a protein with chitinolytic activity. Sequence comparison of the human acidic chitinase and the mouse acidic chitinase revealed an 82% identity and a similarity of 86%. (compare ~~FIGS. 2 and 8~~ Table II (SEQ ID NO:2) and Table III (SEQ ID NO:3)). The catalytic domain of human AMCase is also herewith provided.

[0100] Additional proof for the existence of two discrete genes encoding a phagocyte chitinase (chitotriosidase) and mucosal mucinase/chitinase (AMCase) is rendered by our finding that in man the former enzyme is encoded by a gene in locus 1q31 and the latter by a gene in locus 1p13.

[0101] A mucinase of the invention can be obtained by expression of a nucleic acid encoding the mucinase in a host or host cell and subsequent isolation of the mucinase from the host or host cell or medium in which the host cell is cultured. The host or host cell may be naturally expressing the mucinase. Alternatively, the host or host cell may be genetically engineered. A nucleic acid encoding the mucinase may be provided to the host or host cell.

[0102] A mucinase of the invention can also be obtained by substantially isolating or purifying the mucinase from an environment. Methods for isolating a proteinaceous molecule from an environment are known in the art (for instance, chromatography) and need no further explanation here. A sample comprising the mucinase can, for instance, be enriched for the mucinase by applying the sample onto an affinity column and collecting an elution fraction enriched for the mucinase. Enrichment can also be performed by centrifugation and subsequent separation of a fraction enriched for the mucinase. A person skilled in the art is well capable of performing alternative isolation and/or purification procedures, which are known in the art.

[0103] A nucleic acid encoding a mucinase of the invention, and/or a modified form thereof having a substantially similar mucin-hydrolyzing activity, is suitable for gene therapy. For instance, mammalian cells, preferably cells of a mucosal lining, can be provided with the nucleic acid. After that, the mucinase and/or modified form can be expressed, resulting in (increased) cleavage of mucus. In one embodiment, a cell which is transformed with the nucleic acid does not naturally produce a substantial amount of a mucinase of the invention. However, in another embodiment, the cell already produces the mucinase. In that case, production of mucinase can be enhanced by gene therapy with a nucleic acid of the invention. Gene therapy with a nucleic acid of the invention is, for instance, very suitable for inducing or enhancing mucinase expression in the lungs and/or gastrointestinal tract of a patient suffering from a disease in which mucus is involved.

[0104] Likewise, a nucleic acid encoding a mucinase of the invention which further comprises a chitin-hydrolyzing activity, and/or a modified form thereof having a substantially similar chitin-hydrolyzing activity, is suitable for gene therapy. For instance, mammalian cells, preferably cells of a mucosal lining, can be provided with the nucleic acid, resulting in (enhanced) expression of a mucinase of the invention which further comprises a chitin-hydrolyzing activity. The mucinase is capable of counteracting chitin-containing pathogens present in the mucosal lining.

[0105] The invention will now be illustrated by the following examples which merely serve to exemplify the invention and are not intended to limit the scope of the invention.

EXAMPLES

Example 1. Cloning and composition of cDNAs encoding mouse and human AMCase

Mouse AMCase

[0106] To obtain more insight into the potential occurrence of multiple mammalian chitinases, tissues of mouse and rat were examined for chitinolytic activity using the chitin-like 4-methylumbelliferyl- β -chito-oligosaccharide substrates. In extracts of stomach and intestine, a high level of activity was detected, while extracts of lung, tongue, kidney and plasma showed significant but lower activities. Isoelectric focusing (by flatbed isoelectric focusing in granulated Ultrodex gels (Pharmacia) as described by Renkema et al., 1995) of a mouse lung extract revealed a major peak of chitinolytic activity with pI 4.5 while minor peaks were found with pIs 5.5-6.5 (FIG. 1). Extracts of other mouse and rat tissues showed similar profiles of chitinolytic activity upon isoelectric focusing. The observed rodent chitinase with acidic isoelectric point (pI 4.5 form) differs strikingly from human chitotriosidase, which has an apparent neutral/basic pI.

[0107] The mouse acidic chitinase activity was found to bind to chitin particles with high affinity. Chitin affinity chromatography was used to purify the enzyme. Detergent-free extracts of mouse tissues were prepared by homogenization in 10 volumes of potassium phosphate buffer pH 6.5, using an Ultra-turrax and centrifugation for 20 minutes at 15,000 x g. The mouse intestine extract was adjusted to pH 5.0 by the addition of citric acid (0.2 M); NaCl was added to a final concentration of 2 M. A chitin column was prepared by mixing 10 grams swollen Sepharose G25 fine (Pharmacia, Uppsala, Sweden) with 300 mg of colloidal chitin (prepared as described by Shimahara et al. (Shimahara et al., 1988), followed by equilibration with phosphate-buffered saline (PBS) containing 2M NaCl. The extracts were applied onto the column with a flow speed of 0.4 ml/minute. After extensive washing, bound chitinase was eluted from the column with 8M urea, which was subsequently removed by dialysis. Protein concentrations were determined according to the method of Lowry et al. (Lowry et al 1951) using BSA as a standard. Fractions containing chitinase activity were subjected to SDS-PAGE and Western blotting as described (Renkema et al., 1995).

[0108] The procedure resulted in a 30,000-fold purification of an apparently homogeneous 50 kDa protein. The specific activity of the purified enzyme was 3.9 nmol 4-

methylumbelliferyl-chitotrioside hydrolyzed per mg per hour at pH 5.2, which is almost identical to that of human chitotriosidase.

[0109] The N-terminal amino acid sequence of purified acidic chitinase was determined as described by (Renkema et al., 1995) using a Procise 494 sequencer (Applied Biosystems Perkin Elmer, Foster City, CA, USA) (~~FIG. 2~~Table IV) and was found to be almost identical to that of other known members of the chitinase family. This amino acid sequence allowed the cloning of the corresponding full-length mouse acidic chitinase cDNA, as described in experimental procedures. The full-length cDNA predicts the synthesis of a 50 kDa (pI 4.85) protein with a characteristic signal peptide (~~FIG. 2~~Table IV). Transient expression of this cDNA in COS-1 cells was performed exactly as described previously (Boot et al., 1995) and led to the secretion of a 50 kDa active chitinase with a pI of 4.8.

[0110] Reverse transcription-polymerase chain reaction (RT-PCR) fragments were generated from mouse lung total RNA using degenerate oligonucleotides, as described (Boot et al., 1995). Obtained fragments were cloned in pGEM-T (Promega, Madison, WI, USA), sequenced and compared with the amino acid sequence established by N-terminal protein sequencing. A comparison with the GenBank mouse EST (expressed sequence tag) database using the Basic local alignment search tool (BLAST) at NCBI (National Center for Biotechnology Information) showed that several EST clones matched the mouse chitinase cDNA sequence, for example, ms33h09.y1 (GenBank Accession Number AI892792). This clone was obtained and sequenced. Anti-sense primers were generated complementary to the most 3' region of the EST clone (A-tail primer : 5'- TTTTGGCTACCAATTTTATTGC-3') (SEQ ID NO:5) and two internal anti-sense primers (MAS1 : 5'- CAGCTACAGCAGCAGTAACCATC-3') (SEQ ID NO:6) and (MAS2 : 5'- TTCAGGGATCTCATAGCCAGC-3') (SEQ ID NO:7). The MAS1 and MAS2 primers were used to clone the most 5' end of the mouse acidic chitinase cDNA using 5' rapid amplification of cDNA ends (5' RACE) and the Marathon-Ready mouse Lung cDNA kit (Clontech) according to the instructions of the manufacturer. To obtain the complete coding sequence, a 5' sense primer was generated (MS1 : 5'-CGATGGCCAAGCTACTTCTCGT-3') (SEQ ID NO:8). The total cDNA sequence was subsequently generated using MS1 and the A-tail primer. The fragments of two independent PCR's were cloned into pGEM-T (Promega) and the nucleotide sequence of two independent clones from each PCR were sequenced from both strands by the procedure of Sanger using fluorescent nucleotides on an Applied Biosystems (ABI) 377A automated DNA sequencer following ABI protocols. The

mouse AMCase protein shows considerable sequence homology to human chitotriosidase. Comparison of the amino acid sequence of both mature proteins revealed an identity of 52% and a similarity of 60%. See, Tables V and VI, below.

[0111] Table V. Amino acid sequence of mature (without signal peptide) mouse AMCase (SEQ ID NO:9).

YNLICYFTNWAQYRPGLGSFKPDDINPCLCTHLYAFAG
MQNNEITTIEWNDVTLYKAFNDLKNRNSKLTLLAIGG
WNFGTAPFTTMVSTSONRQTFITSVIKFLROYGFDGLDL
DWEYPGSRGSPPODKHLFTVLVKEMREAFEQEAIESNRP
RLMVTA AVAGGISNIQAGYEIPELSKYLDFIHVMTYDLH
GSWEGYTGENSPLYKYPTETGSNAYLNVDYVMNYWKN
NGAPAEKLIVGFPEYGHFTFILRNPSDNGIGAPTSGDGPGAG
AYTROAGFWAYYEICTFLRSGATEVWDASQEVPIAYK
ANEWLGYDNIKSFVKAQWLKQNNFGGAMIWAIDLDD
FTGSFCDQGKFPLTSTLNKALGISTEGCTAPDVPSEPVTT
PPGSGSGGGSSGGSSGGSGFCADKADGLYPVADDRNAF
WQCINGITYQQHCQAGLVFDTSCNCCNWP

[0112] Table VI. Amino acid sequence of human chitotriosidase (SEQ ID NO:10).

AKLVICYFTNWAQYRQGEARFLPKDLDPCLCTHLYAFA
GMTNHQLSTTEWNDETLYQEFNGLKKMNPCLKTLLAIG
GNFGTQKFTDMVATANNRQTFVNSAIRFLRKYSFDGL
DLDWEYPGSQGSPAVDKERFTTLVODLANAFOQEAQTS
GKERLLLSAAVPAGQTYVDAGYEVDKIAQNLDVFNLM
AYDFHGSWEKVTGHNSPLYKROESGAAASLNVDAAV
QQWLQKGT PASKLILGMPTYGRSFTLASSSDTRVGAPAT
GSGTPGPFTKEGGMLAYYEVCSWKGATKQRIQDQKVP
YIFRDNQWVGFDDEVESFKTKVSYLKQKGLGGAMVWAL
DLDDFAGFSCNQGRYPLIQTLRQELSLPYLPSGTPELEV
KPGQPSEPEHGSPSGQDTFCQGKADGLYPNPRERSSFY
CAAGRLFQQSCPTGLVFSNSCKCCTWN

[0113] Like the human chitotriosidase, the mouse enzyme is predicted to contain an N-terminal catalytic domain of about 39 kDa, a hinge region and a C-terminal chitin binding domain (~~FIG. 2~~see, Table IV, above). The mouse AMCase, like chitotriosidase, is predicted to lack N-linked oligosaccharides, explaining the observed absence of binding to Concanavalin A (data not shown). The apparent molecular masses of identically produced

recombinant human chitotriosidase and recombinant mouse AMCase are comparable when run on an SDS-PAGE gel under reducing conditions. However, under non-reducing conditions, the mouse AMCase migrates significantly slower than the human chitotriosidase (FIG. 43A). Upon gel electrophoresis (under non-reducing conditions) in polyacrylamide gels containing glycolchitin, followed by regeneration of active enzyme and detection of the local digestion of glycolchitin using Calcofluor staining, the mouse AMCase migrates slightly faster than human chitotriosidase (FIG. 43B).

Human AMCase

[0114] It was investigated whether such an acidic chitinase is also present in man. Screening the human EST database at the NCBI with the mouse acidic chitinase cDNA revealed the presence of a highly homologous human EST clone (oq35c04.s1, GenBank Accession Number, AA976830). As mouse AMCase activity was shown in the stomach, the full-length human AMCase cDNA was cloned using human stomach total RNA (Clontech) for the RT-PCR with the same degenerate primers as for the mouse AMCase. A human Marathon-Ready Lung cDNA was used to clone the most 5' end of the cDNA by 5' RACE using the following primers: HAS2 (5'-TCTGACAGCACAGAATCCACTGCC-3') (SEQ ID NO:11) and HAS3-A-tail (5'-TTGACTGCTGATTTTATTGCAG-3') (SEQ ID NO:12). The total cDNA sequence was subsequently generated using HS1 (5'-GCTTTCCAGTCTGGTGGTGAAT-3') (SEQ ID NO:13) and HAS3-Atail. The fragments of two independent PCR's were cloned in pGEM-T (Promega) and sequenced as described above (Table II).

[0115] Screening the GenBank database using the full-length human cDNA revealed that it was almost identical to TSA1902-L (GenBank Accession Number AB025008) and TSA1902-S (GenBank Accession Number AB025009) from a lung cDNA library described by Saito et al. (Saito et al., 1999). These two sequences are most probably splice variants of the human acidic chitinase mRNA. Only expression of full-length human AMCase cDNA in COS-1 cells led to the production of a protein with chitinolytic activity (data not shown). Sequence comparison of the human acidic chitinase (see, Table VII, below) and the mouse acidic chitinase (see, Table V, above) revealed an 82% identity and a similarity of 86% (~~FIG. 8B~~).

[0116] **Table VII.** Amino acid sequence of mature (without signal peptide) human AMCase (SEQ ID NO:14).

YQLTCYFTNWAQYRPGGLGRFMPDNIDPCLCTHLIYAFA
GRQNEITTIEWNDVTLYQAFNGLKNKNSQLKTLLAIGG
WNFGTAPFTAMVSTPENROTFITSVIKFLRQYEFDGLDF
DWEYPGSRGSPPODKHLFTVLVQEMREAFEQEAKQINK
PRLMVTAAVAAGISNIQSGYEIPQLSQYLDYIHVMTYDL
HGSWEGYTGENSPLYKYPTDTGSNAYLNVDYVMNYW
KDNGAPAEKLIVGFPTYGHNFILSNPSNTGIGAPTSAGP
AGPYAKESGIWAYYEICTFLKNGATQGWDAPQEVPIAY
QGNVWVGYDNIKSFDIKAQWLKHNKFGGAMVWIDL
DDFTGTFCNOGKFPLISTLKKALGLOSASCTAPAOPIEPIT
AAPSGSGNGSGSSSSGGSSGGSGFCAVRANGLYPVANN
RNAFWHCVNGVTYQQNCQAGLVFDTSCDCCNWA

[0117] The demonstration by Saito et al. that the gene encoding TSA1902 is located on chromosome 1p13 (Saito et al., 1999) indicates that mammals contain indeed at least two discrete genes that encode functional chitinases, being chitotriosidase (locus 1q32) and AMCase (locus 1p13).

Example 2. Tissue expression of human and mouse AMCase RNA

[0118] Another major difference between human chitotriosidase and the mouse AMCase is revealed by comparison of RNA expression patterns. Total RNA was isolated using RNazol B (Biosolve, Barneveld, The Netherlands) according to the instructions of the manufacturer. Northern blots, using 15 µg of total RNA, were performed as described (Boot et al., 1995). Mouse RNA Master Blots (Clontech, Palo Alto, CA, USA) were used to examine the tissue distribution of transcripts according to the instructions of the manufacturer. The following probes were used: the full-length mouse acidic chitinase cDNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as control. Radiolabeling and hybridization was conducted as described previously (Boot et al., 1995). Quantification of radioactivity was performed using a phosphor imager (Storm phosphor imager, Molecular Dynamics, Sunnyvale, CA, USA).

[0119] Whereas human chitotriosidase mRNA is mainly found in lymph node, bone marrow and lung, the mouse AMCase mRNA is predominantly found in, of the screened tissues, stomach, submaxillary gland and, at a lower level, in the lung (FIG. 65). Surprisingly, no mouse acidic chitinase mRNA could be detected in the small intestine. This can be explained by an absence of mRNA, or by mRNA levels in the sample that were too low for detection with the technique used. These results suggest that the protein in the

intestine is probably derived from the upper parts of the gastrointestinal tract, such as the stomach.

[0120] In rat tissues, a comparable acidic chitinase was observed. Our findings indicate that the acidic chitinase in rodents is distinct from human chitotriosidase. The discrete enzyme is therefore referred to as acidic mammalian chitinase or AMCase.

[0121] Next, the tissue distribution of this human mRNA was examined. Total RNA was isolated as described above and human RNA Master Blots (Clontech, Palo Alto, CA, USA) were probed with the human EST clone oq35c04.s1 (GenBank Accession Number AA976830) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as control to examine the tissue distribution as described above.

[0122] The expression pattern of the human AMCase mRNA is similar to the expression pattern of the mouse acidic chitinase (FIGS. 65, 76), being highly expressed in the stomach and, at a lower level, in the lung, as far as the RNA master blots are representative for these tissues. Expression in additional tissues that were not tested cannot be excluded.

Example 3. Degradation of chitin by AMCase

[0123] Several different assays revealed that the mouse acidic chitinase is able to degrade chitin and, therefore, has to be considered to be a true chitinase. Crab shell chitin (Poly-[1-4]- β -D-N-acetylglucosamine, Sigma) was used as a natural substrate to determine chitinase activity as described (Renkema et al., 1997). The chitin fragments were analyzed by fluorophore-assisted carbohydrate electrophoresis (FACE) as described by Jackson (Jackson 1990). FACE analysis revealed that recombinant mouse chitinase, like chitotriosidase, releases mainly chitobioside fragments from chitin (FIG. 32). Chitinase enzyme activity was determined in another assay with the fluorogenic substrates 4MU-chitobiose (4-methylumbelliferyl β -D-N,N'-diacetylchitobiose, Sigma, St Louis, USA) and 4MU-chitotriose (4-methylumbelliferyl β -D-N,N',N''-triacetylchitotriose, Sigma). Assay mixtures contained 0.027 mM substrate and 1 mg/ml of bovine serum albumin (BSA) in McIlvaine buffer (100 mM citric acid, 200mM sodium phosphate) at the indicated pH. The standard enzyme activity assay for human chitotriosidase with 4MU-chitotriose substrate was performed at pH 5.2, as previously described (Hollak et al., 1994). The standard AMCase enzyme activity assays with 4MU-chitobiose substrate were performed at pH 4.5. Like chitotriosidase and some other non-mammalian chitinases, the mouse acidic chitinase activity in this assay is strongly inhibited (IC_{50} of 0.4 μ M) by the competitive chitinase inhibitor

allosamidin (Milewski et al., 1992, Dickinson et al., 1989, McNab and Glover 1991). Measurements of chitin formation during regeneration of fungal spheroplasts were performed as described by Hector and Braun (Hector and Braun 1986). Briefly, spheroplasts were prepared from the *Candida albicans* strain CAi-4 (*ura3*), grown overnight in YPD medium (1% yeast extract, 2% peptone, 2% glucose) at 28°C. Cells were concentrated by centrifugation and incubated with 2.5 mg/ml zymolyase (100T, ICN Immuno Biologicals, Costa Mesa, CA, USA) in buffer containing 50 mM sodium phosphate pH 7.5, 1.2 M sorbitol and 27 mM β -mercaptoethanol for 60 minutes at 37°C. After extensive washing, spheroplasts were allowed to regenerate in 96-well microtiter plates in regeneration buffer (0.25% (w/v) MES buffer pH 6.7, containing 0.17% (w/v) Yeast Nitrogen Base (without amino acids and ammonium sulfate, Sigma), 0.15% (w/v) ammonium sulfate, 2% (w/v) glucose, 1.2 M sorbitol, 20 μ g/ml uridine) at 37 °C. Chitinase enzyme preparations were added in 3 μ g/ml. After a 2 hour incubation, 50 μ l of 300 μ g/ml Calcofluor white (Sigma) in 10 mM sodium phosphate buffer pH 7.5 containing 1.2 M sorbitol was added. After 5 minutes, the plates were washed with buffer only and fluorescence was determined using an LS 50 Perkin Elmer fluorometer (excitation 405 nm, emission 450 nm).

[0124] In addition, the mouse acidic chitinase and chitotriosidase were both able to digest chitin in the cell wall of regenerating spheroplasts of *Candida albicans*. The chitin content of the cell wall was determined with the Calcofluor white stain. When regenerating cells were incubated for 2 hours with 3 μ g per ml recombinant chitotriosidase or 3 μ g per ml recombinant mouse acidic chitinase, the chitin content was reduced by 27% and 33%, respectively. Concomitant presence of allosamidin during the incubation completely abolished the effect of both recombinant chitinases.

[0125] Finally, incubation of hyphae of *Candida albicans* with recombinant 50 kDa or 39 kDa AMCase as described by Boot et al. (1995) results in selective lysis of the growing tip, showing a fungistatic effect of AMCase similar to the effect of chitotriosidase described by Boot et al. (1995).

Example 4. pH optimum of chitinase activity of AMCase

[0126] A striking difference between chitotriosidase and AMCase is their behavior at acidic pH.

[0127] The pH dependence of chitinase activity of AMCase was determined by incubating purified enzyme at a different pH (McIlvaine buffer range 1.7-8.5) with 4-

methylumbelliferyl substrates. Release of the fluorescent 4-methylumbelliferone was monitored fluorometrically (excitation 445 nm and emission 360 nm). The results are presented in FIG. 54A. The mouse acidic chitinase shows a pronounced pH optimum at pH 2.3 and a less pronounced optimum at a more neutral pH (pH 4.7). Chitotriosidase, however, shows only a broad pH optimum (see see, FIG. 54A) and is completely inactivated by pre-incubation at low pH (see see, FIG. 54B). In the presence of 0.5% (w/v) trichloroacetic acid (TCA), 58% of chitotriosidase is precipitated, while under similar circumstances, the mouse acidic chitinase remains in solution. At 2.5% (w/v) TCA all chitotriosidase precipitates while 26% of mouse acidic chitinase remains unprecipitated (FIG. 54C).

Example 5. Degradation of mammalian mucin by AMCase

[0128] The effect of AMCase on glycoproteins was studied by evaluating the effect of AMCase on mucin, a glycoprotein expressed on mucosal surfaces in the airways and gastrointestinal tract. Bovine submaxillary gland mucin (Sigma) 125 µg was dissolved in 40 µl 0.05M NaAc pH 5.0 in the presence or absence of 500 ng mouse AMCase. After overnight incubation at 37°C, the mucin was electrophorized on an SDS-PAGE gel (7.5% homogeneous, followed by silver staining). As is shown in FIG. 97, the mucin was degraded by treatment with the AMCase. This shows that AMCase is an endogenous regulator of mucus viscosity that can be employed in diseases in which overproduction or high viscosity of mucus is involved. Similar experiments may be performed using human AMCase to demonstrate its mucinolytic activity.

[0129] Experiments can be performed as described by Puchelle et al. (1996) to demonstrate that AMCase is capable of decreasing the viscosity of cystic fibrosis sputum and sputum derived from chronic obstructive pulmonary disease patients.

Example 6. pH optimum of mucin hydrolyzing activity of AMCase

[0130] The pH dependence of the mucin hydrolyzing activity of AMCase is determined by incubating purified enzyme at a different pH (for example, in McIlvaine buffer range 1.7-8.5) with mammalian mucin (see Example 5).

Example 7. Generation of antibodies to AMCase

[0131] Polyclonal antiserum against AMCase is raised by immunization of rabbits with recombinant human AMCase (*e.g.*, 4 injections/animal; 20-200 µg/injection). After the final booster, the animals are bled to determine the titer of the polyclonal antiserum.

[0132] To obtain monoclonal antibodies, 6-8 wk old Balbic mice are immunized with recombinant human AMCase (for example, 4 times with 2 wk intervals with 10-100 µg/injection dissolved in Freund's complete adjuvans for the first injection, and Freund's incomplete adjuvans for subsequent immunizations). Splenocytes are isolated and fused with a fusion cell line such as Sp2/0 myeloma cells, followed by limiting dilution. Growing clones are screened using, for example, an enzyme-linked immunosorbent assay (ELISA). Therefore, 96-well plates are coated with recombinant human AMCase or with a control protein. The culture supernatant is added, followed by washing and the addition of a labeled anti-mouse antibody for detection. After limited dilution cloning of AMCase-specific antibody-producing hybridomas, stable hybridomas are obtained. From each clone cell, supernatant is collected, and by affinity chromatography using protein A sepharose columns (Pharmacia, Uppsala, Sweden) monoclonal antibodies are purified.

Example 8. A quantitative assay to detect AMCase

[0133] Using the AMCase-specific antibodies of Example 7 and recombinant human AMCase, a quantitative assay for the detection of AMCase is set up. The assay is used to quantify AMCase. In addition, the assay is able to differentiate between chitotriosidase and AMCase.

Example 9. Anti-fungal activity of AMCase in animal models

[0134] Allergic bronchopulmonary aspergillosis is a complication that is often seen in CF and asthma patients. As AMCase has an effect on mucus as well as *Aspergillus*, proof of concept is obtained in a lung infection model with *Aspergillus*, in which mouse AMCase is administered intratracheally.

Example 10. Mucolytic activity of AMCase in a mouse model for cystic fibrosis

[0135] CFTR-knockout mice are currently the only relevant disease model for human CF. The mice have the same genetic defect as CF patients. CFTR-knockout mice display many characteristics of intestinal disease in CF but fail to develop respiratory

infections or other signs of overt lung disease. To prevent massive mortality in the *CFTR*-knockout mice by intestinal obstruction, animals need to be fed a liquid diet during the weaning period. Interestingly, *CFTR*-knockout mice that also are deficient for MUC1 have a much better survival on a solid diet, indicating an important role for mucus formation in the intestine of the *CFTR*-knockout mice. Clearly, the model has its limitations for studying the effect of AMCase; however, it is a good model on which to study the effect of AMCase on gastrointestinal disease seen in CF.

Example 11. Treatment of mucin-associated human diseases

[0136] Treatment of diseases that are typically associated with the undue, inappropriate or excessive production, or the insufficient removal of mucous are treated or ameliorated with mucinase. These diseases include but are not limited to COPD and CF. In the case of CF, the indication for use of the mucinase is for both the digestive tract as well as the pulmonary signs and symptoms of excessive amounts of mucous present. Administration routes of the mucinase are such that the highest effective dose can be administered to the relevant anatomical area indicated for mucinolytic treatment.

Example 12. Treatment of topical infections by chitin-containing pathogens

[0137] A variety and myriad number of dermatological and pulmonary tract fungal infections are deemed to be indications for treatment with a mucinase of the invention.

[0138] These include but are not limited to the *Dermatophytoses (Tinea's)*, *Candidiasis*, *Aspergillosis*, *Mucomycosis* and *Pneumocystis carinii*. Patients presenting with dermatological manifestations of fungal disease can be treated by the regular application of a topical formulation of a mucinase of the invention appropriate for the relevant anatomical region infected.

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Ser Phe Asp Ile Lys Ala Gln Trp Leu Lys His Asn Lys Phe Gly Gly	
345 350 355	
gcc atg gtc tgg gcc att gat ctg gat gac ttc act ggc act ttc tgc	1219
Ala Met Val Trp Ala Ile Asp Leu Asp Asp Phe Thr Gly Thr Phe Cys	

360	365	370	
aac cag ggc aag ttt ccc cta atc tcc acc ctg aag aag gcc ctc ggc			1267
Asn Gln Gly Lys Phe Pro Leu Ile Ser Thr Leu Lys Lys Ala Leu Gly			
375	380	385	
ctg cag agt gca agt tgc acg gct cca gct cag ccc att gag cca ata			1315
Leu Gln Ser Ala Ser Cys Thr Ala Pro Ala Gln Pro Ile Glu Pro Ile			
390	395	400	
act gct gct ccc agt ggc agc ggg aac ggg agc ggg agt agc agc tct			1363
Thr Ala Ala Pro Ser Gly Ser Gly Asn Gly Ser Gly Ser Ser Ser Ser			
405	410	415	420
gga gcc agc tcg gga gcc agt gga ttc tgt gct gtc aga gcc aac gcc			1411
Gly Gly Ser Ser Gly Gly Ser Gly Phe Cys Ala Val Arg Ala Asn Gly			
425	430	435	
ctc tac ccc gtg gca aat aac aga aat gcc ttc tgg cac tgc gtg aat			1459
Leu Tyr Pro Val Ala Asn Asn Arg Asn Ala Phe Trp His Cys Val Asn			
440	445	450	
gga gtc acg tac cag cag aac tgc cag gcc ggg ctt gtc ttc gac acc			1507
Gly Val Thr Tyr Gln Gln Asn Cys Gln Ala Gly Leu Val Phe Asp Thr			
455	460	465	
agc tgt gat tgc tgc aac tgg gca taaacctgac ctggtctata ttcctagag			1561
Ser Cys Asp Cys Cys Asn Trp Ala			
470	475		
ttccagtctc ttttgcttag gacatgttgc ccctacctaa agtcctgcaa taaaatcagc			1621
agtc			1625

<210> 3

<211> 1525

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Combined DNA/RNA Molecule: mouse
AMCase cDNA sequence and deduced amino acid
sequence

<220>

<223> Description of Artificial Sequence: mouse AMCase
cDNA sequence and deduced amino acid sequence

<220>

<221> CDS

<222> (1)..(1419)

<400> 3

atg gcc aag cta ctt ctc gtc aca ggt ctg gct ctt ctg ctg aat gct	48
Met Ala Lys Leu Leu Val Thr Gly Leu Ala Leu Leu Asn Ala	
1	5
10	15

cag ctg ggg tct gcc tac aat ctg ata tgc tat ttc acc aac tgg gcc	96
Gln Leu Gly Ser Ala Tyr Asn Leu Ile Cys Tyr Phe Thr Asn Trp Ala	
20 25 30	
cag tat cgg cca ggt ctg ggg agc ttc aag cct gat gac att aac ccc	144
Gln Tyr Arg Pro Gly Leu Gly Ser Phe Lys Pro Asp Asp Ile Asn Pro	
35 40 45	
tgc ctg tgt act cac ctg atc tat gcc ttt gct ggg atg cag aac aat	192
Cys Leu Cys Thr His Leu Ile Tyr Ala Phe Ala Gly Met Gln Asn Asn	
50 55 60	
gag atc acc acc ata gaa tgg aat gat gtt act ctc tat aaa gct ttc	240
Glu Ile Thr Thr Ile Glu Trp Asn Asp Val Thr Leu Tyr Lys Ala Phe	
65 70 75 80	
aat gac ttg aaa aac agg aac agc aaa ctg aaa acc ctc ctg gca att	288
Asn Asp Leu Lys Asn Arg Asn Ser Lys Leu Lys Thr Leu Leu Ala Ile	
85 90 95	
gga ggc tgg aac ttt gga act gct cct ttc act acc atg gtt tcc act	336
Gly Gly Trp Asn Phe Gly Thr Ala Pro Phe Thr Thr Met Val Ser Thr	
100 105 110	
tct cag aac cgc cag acc ttc att acc tca gtc atc aaa ttt ctg cgt	384
Ser Gln Asn Arg Gln Thr Phe Ile Thr Ser Val Ile Lys Phe Leu Arg	
115 120 125	
cag tat ggg ttt gat gga ctg gac ctg gac tgg gaa tac cca ggc tca	432
Gln Tyr Gly Phe Asp Gly Leu Asp Leu Asp Trp Glu Tyr Pro Gly Ser	
130 135 140	
cgt ggg agc cct cct cag gac aag cat ctc ttc act gtc ctg gtg aag	480
Arg Gly Ser Pro Pro Gln Asp Lys His Leu Phe Thr Val Leu Val Lys	
145 150 155 160	
gaa atg cgt gaa gct ttt gag cag gag gct att gag agc aac agg ccc	528
Glu Met Arg Glu Ala Phe Glu Gln Glu Ala Ile Glu Ser Asn Arg Pro	
165 170 175	
aga ctg atg gtt act gct gct gta gct ggt ggg att tcc aac atc cag	576
Arg Leu Met Val Thr Ala Ala Val Ala Gly Gly Ile Ser Asn Ile Gln	
180 185 190	
gct ggc tat gag atc cct gaa ctt tct aag tac ctg gat ttc atc cat	624
Ala Gly Tyr Glu Ile Pro Glu Leu Ser Lys Tyr Leu Asp Phe Ile His	
195 200 205	
gtc atg aca tat gac ctc cat ggc tcc tgg gag ggc tac act ggg gag	672
Val Met Thr Tyr Asp Leu His Gly Ser Trp Glu Gly Tyr Thr Gly Glu	
210 215 220	
aat agt cct ctt tac aaa tac cct act gag act ggt agc aat gcc tac	720
Asn Ser Pro Leu Tyr Lys Tyr Pro Thr Glu Thr Gly Ser Asn Ala Tyr	
225 230 235 240	
ctc aat gtg gat tat gtc atg aac tat tgg aag aac aat gga gcc cca	768
Leu Asn Val Asp Tyr Val Met Asn Tyr Trp Lys Asn Asn Gly Ala Pro	

245	250	255	
gct gag aag ctc att gtt gga ttc cca gag tat gga cac acc ttc atc	816		
Ala Glu Lys Leu Ile Val Gly Phe Pro Glu Tyr Gly His Thr Phe Ile			
260	265	270	
ctg aga aac ccc tct gat aat gga att ggt gcc cct acc tct ggt gat	864		
Leu Arg Asn Pro Ser Asp Asn Gly Ile Gly Ala Pro Thr Ser Gly Asp			
275	280	285	
ggc cct gct ggc gcc tat acc aga cag gct ggg ttc tgg gcc tac tat	912		
Gly Pro Ala Gly Ala Tyr Thr Arg Gln Ala Gly Phe Trp Ala Tyr Tyr			
290	295	300	
gag att tgc acc ttt ctg aga agt gga gcc act gag gtc tgg gat gcc	960		
Glu Ile Cys Thr Phe Leu Arg Ser Gly Ala Thr Glu Val Trp Asp Ala			
305	310	315	320
tcc caa gaa gtg ccc tat gcc tat aag gcc aac gag tgg ctt ggc tat	1008		
Ser Gln Glu Val Pro Tyr Ala Tyr Lys Ala Asn Glu Trp Leu Gly Tyr			
325	330	335	
gac aat atc aag agc ttc agt gtt aag gct cag tgg ctt aag cag aac	1056		
Asp Asn Ile Lys Ser Phe Ser Val Lys Ala Gln Trp Leu Lys Gln Asn			
340	345	350	
aat ttt gga ggt gcc atg atc tgg gcc att gac ctt gat gac ttc act	1104		
Asn Phe Gly Gly Ala Met Ile Trp Ala Ile Asp Leu Asp Asp Phe Thr			
355	360	365	
ggc tct ttc tgt gat cag gga aaa ttt cct ctg act tct act ttg aac	1152		
Gly Ser Phe Cys Asp Gln Gly Lys Phe Pro Leu Thr Ser Thr Leu Asn			
370	375	380	
aaa gcc ctt ggc ata tcc act gaa ggt tgc aca gct cct gac gtg cct	1200		
Lys Ala Leu Gly Ile Ser Thr Glu Gly Cys Thr Ala Pro Asp Val Pro			
385	390	395	400
tcc gag cca gtg act act cct cca gga agt ggg agt ggg ggt gga agc	1248		
Ser Glu Pro Val Thr Thr Pro Pro Gly Ser Gly Ser Gly Gly Gly Ser			
405	410	415	
tcc gga gga agc tct gga ggc agt gga ttc tgt gcc gac aaa gca gat	1296		
Ser Gly Gly Ser Ser Gly Gly Ser Gly Phe Cys Ala Asp Lys Ala Asp			
420	425	430	
ggc ctc tac cct gtg gca gat gac aga aat gct ttt tgg cag tgc atc	1344		
Gly Leu Tyr Pro Val Ala Asp Asp Arg Asn Ala Phe Trp Gln Cys Ile			
435	440	445	
aat gga atc aca tac cag cag cat tgt caa gca ggg ctt gtt ttt gat	1392		
Asn Gly Ile Thr Tyr Gln Gln His Cys Gln Ala Gly Leu Val Phe Asp			
450	455	460	
acc agc tgt aat tgc tgc aac tgg cca tgaacctaat gccattcttc	1439		
Thr. Ser Cys Asn Cys Asn Trp Pro			
465	470		

cagaaatttc tgcactctcc ttactcctc accaaaagta actatcttcc cttaacctt 1499

atgcaataaaa attggtagcc aaaaca 1525

<210> 4

<211> 473

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: mouse AMCase
amino acid sequence deduced from cDNA sequence

<400> 4

Met Ala Lys Leu Leu Leu Val Thr Gly Leu Ala Leu Leu Leu Asn Ala
1 5 10 15

Gln Leu Gly Ser Ala Tyr Asn Leu Ile Cys Tyr Phe Thr Asn Trp Ala
20 25 30

Gln Tyr Arg Pro Gly Leu Gly Ser Phe Lys Pro Asp Asp Ile Asn Pro
35 40 45

Cys Leu Cys Thr His Leu Ile Tyr Ala Phe Ala Gly Met Gln Asn Asn
50 55 60

Glu Ile Thr Thr Ile Glu Trp Asn Asp Val Thr Leu Tyr Lys Ala Phe
65 70 75 80

Asn Asp Leu Lys Asn Arg Asn Ser Lys Leu Lys Thr Leu Leu Ala Ile
85 90 95

Gly Gly Trp Asn Phe Gly Thr Ala Pro Phe Thr Thr Met Val Ser Thr
100 105 110

Ser Gln Asn Arg Gln Thr Phe Ile Thr Ser Val Ile Lys Phe Leu Arg
115 120 125

Gln Tyr Gly Phe Asp Gly Leu Asp Leu Asp Trp Glu Tyr Pro Gly Ser
130 135 140

Arg Gly Ser Pro Pro Gln Asp Lys His Leu Phe Thr Val Leu Val Lys
145 150 155 160

Glu Met Arg Glu Ala Phe Glu Gln Glu Ala Ile Glu Ser Asn Arg Pro
165 170 175

Arg Leu Met Val Thr Ala Ala Val Ala Gly Gly Ile Ser Asn Ile Gln
180 185 190

Ala Gly Tyr Glu Ile Pro Glu Leu Ser Lys Tyr Leu Asp Phe Ile His
195 200 205

Val Met Thr Tyr Asp Leu His Gly Ser Trp Glu Gly Tyr Thr Gly Glu
210 215 220

Asn Ser Pro Leu Tyr Lys Tyr Pro Thr Glu Thr Gly Ser Asn Ala Tyr
225 230 235 240

57

Leu	Asn	Val	Asp	Tyr	Val	Met	Asn	Tyr	Trp	Lys	Asn	Asn	Gly	Ala	Pro		
				245				250				255					
<hr/>																	
Ala	Glu	Lys	Leu	Ile	Val	Gly	Phe	Pro	Glu	Tyr	Gly	His	Thr	Phe	Ile		
				260				265				270					
<hr/>																	
Leu	Arg	Asn	Pro	Ser	Asp	Asn	Gly	Ile	Gly	Ala	Pro	Thr	Ser	Gly	Asp		
				275				280				285					
<hr/>																	
Gly	Pro	Ala	Gly	Ala	Tyr	Thr	Arg	Gln	Ala	Gly	Phe	Trp	Ala	Tyr	Tyr		
				290				295				300					
<hr/>																	
Glu	Ile	Cys	Thr	Phe	Leu	Arg	Ser	Gly	Ala	Thr	Glu	Val	Trp	Asp	Ala		
305								310				315				320	
<hr/>																	
Ser	Gln	Glu	Val	Pro	Tyr	Ala	Tyr	Lys	Ala	Asn	Glu	Trp	Leu	Gly	Tyr		
				325				330				335					
<hr/>																	
Asp	Asn	Ile	Lys	Ser	Phe	Ser	Val	Lys	Ala	Gln	Trp	Leu	Lys	Gln	Asn		
				340				345				350					
<hr/>																	
Asn	Phe	Gly	Gly	Ala	Met	Ile	Trp	Ala	Ile	Asp	Leu	Asp	Asp	Phe	Thr		
				355				360				365					
<hr/>																	
Gly	Ser	Phe	Cys	Asp	Gln	Gly	Lys	Phe	Pro	Leu	Thr	Ser	Thr	Leu	Asn		
370								375				380					
<hr/>																	
Lys	Ala	Leu	Gly	Ile	Ser	Thr	Glu	Gly	Cys	Thr	Ala	Pro	Asp	Val	Pro		
385								390				395				400	
<hr/>																	
Ser	Glu	Pro	Val	Thr	Thr	Pro	Pro	Gly	Ser	Gly	Ser	Gly	Gly	Gly	Ser		
				405				410				415					
<hr/>																	
Ser	Gly	Gly	Ser	Ser	Gly	Gly	Ser	Gly	Phe	Cys	Ala	Asp	Lys	Ala	Asp		
				420				425				430					
<hr/>																	
Gly	Leu	Tyr	Pro	Val	Ala	Asp	Asp	Arg	Asn	Ala	Phe	Trp	Gln	Cys	Ile		
435								440				445					
<hr/>																	
Asn	Gly	Ile	Thr	Tyr	Gln	Gln	His	Cys	Gln	Ala	Gly	Leu	Val	Phe	Asp		
450								455				460					
<hr/>																	
Thr	Ser	Cys	Asn	Cys	Cys	Asn	Trp	Pro									
465				470													

<210> 5

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: A-tail primer

<220>

<221> misc feature

<222> (1)..(22)

<400> 5
ttttggctac caattttatt gc 22

<210> 6
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: anti-sense
primer MAS1

<220>
<221> misc feature
<222> (1)..(23)

<400> 6
cagctacagc agcagtaacc atc 23

<210> 7
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: anti-sense
primer MAS2

<220>
<221> misc feature
<222> (1)..(21)

<400> 7
ttcagggatc tcatagccag c 21

<210> 8
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: sense primer
MS1

<220>
<221> misc feature
<222> (1)..(22)

<400> 8
cgatggccaa gctacttctc gt 22

<210> 9
<211> 452
<212> PRT

<213> Mus musculus

<220>

<221> SITE

<222> (1)..(452)

<223> /note="Mouse AMCase"

<400> 9

Tyr	Asn	Leu	Ile	Cys	Tyr	Phe	Thr	Asn	Trp	Ala	Gln	Tyr	Arg	Pro	Gly
1				5					10					15	

Leu	Gly	Ser	Phe	Lys	Pro	Asp	Asp	Ile	Asn	Pro	Cys	Leu	Cys	Thr	His
			20					25						30	

Leu	Ile	Tyr	Ala	Phe	Ala	Gly	Met	Gln	Asn	Asn	Glu	Ile	Thr	Thr	Ile
		35					40						45		

Glu	Trp	Asn	Asp	Val	Thr	Leu	Tyr	Lys	Ala	Phe	Asn	Asp	Leu	Lys	Asn
	50					55					60				

Arg	Asn	Ser	Lys	Leu	Lys	Thr	Leu	Leu	Ala	Ile	Gly	Gly	Trp	Asn	Phe
65					70					75					80

Gly	Thr	Ala	Pro	Phe	Thr	Thr	Met	Val	Ser	Thr	Ser	Gln	Asn	Arg	Gln
				85				90						95	

Thr	Phe	Ile	Thr	Ser	Val	Ile	Lys	Phe	Leu	Arg	Gln	Tyr	Gly	Phe	Asp
			100					105					110		

Gly	Leu	Asp	Leu	Asp	Trp	Glu	Tyr	Pro	Gly	Ser	Arg	Gly	Ser	Pro	Pro
		115					120					125			

Gln	Asp	Lys	His	Leu	Phe	Thr	Val	Leu	Val	Lys	Glu	Met	Arg	Glu	Ala
	130						135					140			

Phe	Glu	Gln	Glu	Ala	Ile	Glu	Ser	Asn	Arg	Pro	Arg	Leu	Met	Val	Thr
145					150					155					160

Ala	Ala	Val	Ala	Gly	Gly	Ile	Ser	Asn	Ile	Gln	Ala	Gly	Tyr	Glu	Ile
				165					170					175	

Pro	Glu	Leu	Ser	Lys	Tyr	Leu	Asp	Phe	Ile	His	Val	Met	Thr	Tyr	Asp
		180						185					190		

Leu	His	Gly	Ser	Trp	Glu	Gly	Tyr	Thr	Gly	Glu	Asn	Ser	Pro	Leu	Tyr
	195						200					205			

Lys	Tyr	Pro	Thr	Glu	Thr	Gly	Ser	Asn	Ala	Tyr	Leu	Asn	Val	Asp	Tyr
	210					215					220				

Val	Met	Asn	Tyr	Trp	Lys	Asn	Asn	Gly	Ala	Pro	Ala	Glu	Lys	Leu	Ile
225					230					235					240

Val	Gly	Phe	Pro	Glu	Tyr	Gly	His	Thr	Phe	Ile	Leu	Arg	Asn	Pro	Ser
				245					250					255	

Asp	Asn	Gly	Ile	Gly	Ala	Pro	Thr	Ser	Gly	Asp	Gly	Pro	Ala	Gly	Ala
		260						265					270		

Tyr Thr Arg Gln Ala Gly Phe Trp Ala Tyr Tyr Glu Ile Cys Thr Phe
275 280 285
Leu Arg Ser Gly Ala Thr Glu Val Trp Asp Ala Ser Gln Glu Val Pro
290 295 300
Tyr Ala Tyr Lys Ala Asn Glu Trp Leu Gly Tyr Asp Asn Ile Lys Ser
305 310 315 320
Phe Ser Val Lys Ala Gln Trp Leu Lys Gln Asn Asn Phe Gly Gly Ala
325 330 335
Met Ile Trp Ala Ile Asp Leu Asp Asp Phe Thr Gly Ser Phe Cys Asp
340 345 350
Gln Gly Lys Phe Pro Leu Thr Ser Thr Leu Asn Lys Ala Leu Gly Ile
355 360 365
Ser Thr Glu Gly Cys Thr Ala Pro Asp Val Pro Ser Glu Pro Val Thr
370 375 380
Thr Pro Pro Gly Ser Gly Ser Gly Gly Gly Ser Ser Gly Gly Ser Ser
385 390 395 400
Gly Gly Ser Gly Phe Cys Ala Asp Lys Ala Asp Gly Leu Tyr Pro Val
405 410 415
Ala Asp Asp Arg Asn Ala Phe Trp Gln Cys Ile Asn Gly Ile Thr Tyr
420 425 430
Gln Gln His Cys Gln Ala Gly Leu Val Phe Asp Thr Ser Cys Asn Cys
435 440 445
Cys Asn Trp Pro
450

<210> 10
 <211> 445
 <212> PRT
 <213> Homo sapiens

<220>
 <221> SITE
 <222> (1)..(445)
 <223> /note="Human chitotriosidase"

<400> 10
Ala Lys Leu Val Cys Tyr Phe Thr Asn Trp Ala Gln Tyr Arg Gln Gly
1 5 10 15
Glu Ala Arg Phe Leu Pro Lys Asp Leu Asp Pro Ser Leu Cys Thr His
20 25 30
Leu Ile Tyr Ala Phe Ala Gly Met Thr Asn His Gln Leu Ser Thr Thr
35 40 45

Glu Trp Asn Asp Glu Thr Leu Tyr Gln Glu Phe Asn Gly Leu Lys Lys			
50	55	60	
Met Asn Pro Lys Leu Lys Thr Leu Leu Ala Ile Gly Gly Trp Asn Phe			
65	70	75	80
Gly Thr Gln Lys Phe Thr Asp Met Val Ala Thr Ala Asn Asn Arg Gln			
	85	90	95
Thr Phe Val Asn Ser Ala Ile Arg Phe Leu Arg Lys Tyr Ser Phe Asp			
	100	105	110
Gly Leu Asp Leu Asp Trp Glu Tyr Pro Gly Ser Gln Gly Ser Pro Ala			
	115	120	125
Val Asp Lys Glu Arg Phe Thr Thr Leu Val Gln Asp Leu Ala Asn Ala			
	130	135	140
Phe Gln Gln Glu Ala Gln Thr Ser Gly Lys Glu Arg Leu Leu Leu Ser			
145	150	155	160
Ala Ala Val Pro Ala Gly Gln Thr Tyr Val Asp Ala Gly Tyr Glu Val			
	165	170	175
Asp Lys Ile Ala Gln Asn Leu Asp Phe Val Asn Leu Met Ala Tyr Asp			
	180	185	190
Phe His Gly Ser Trp Glu Lys Val Thr Gly His Asn Ser Pro Leu Tyr			
	195	200	205
Lys Arg Gln Glu Glu Ser Gly Ala Ala Ala Ser Leu Asn Val Asp Ala			
	210	215	220
Ala Val Gln Gln Trp Leu Gln Lys Gly Thr Pro Ala Ser Lys Leu Ile			
225	230	235	240
Leu Gly Met Pro Thr Tyr Gly Arg Ser Phe Thr Leu Ala Ser Ser Ser			
	245	250	255
Asp Thr Arg Val Gly Ala Pro Ala Thr Gly Ser Gly Thr Pro Gly Pro			
	260	265	270
Phe Thr Lys Glu Gly Gly Met Leu Ala Tyr Tyr Glu Val Cys Ser Trp			
	275	280	285
Lys Gly Ala Thr Lys Gln Arg Ile Gln Asp Gln Lys Val Pro Tyr Ile			
	290	295	300
Phe Arg Asp Asn Gln Trp Val Gly Phe Asp Asp Val Glu Ser Phe Lys			
305	310	315	320
Thr Lys Val Ser Tyr Leu Lys Gln Lys Gly Leu Gly Gly Ala Met Val			
	325	330	335
Trp Ala Leu Asp Leu Asp Asp Phe Ala Gly Phe Ser Cys Asn Gln Gly			
	340	345	350
Arg Tyr Pro Leu Ile Gln Thr Leu Arg Gln Glu Leu Ser Leu Pro Tyr			

355	360	365
Leu Pro Ser Gly Thr Pro Glu Leu Glu Val Pro Lys Pro Gly Gln Pro		
370	375	380
Ser Glu Pro Glu His Gly Pro Ser Pro Gly Gln Asp Thr Phe Cys Gln		
385	390	395
Gly Lys Ala Asp Gly Leu Tyr Pro Asn Pro Arg Glu Arg Ser Ser Phe		
405	410	415
Tyr Ser Cys Ala Ala Gly Arg Leu Phe Gln Gln Ser Cys Pro Thr Gly		
420	425	430
Leu Val Phe Ser Asn Ser Cys Lys Cys Cys Thr Trp Asn		
435	440	445

<210> 11
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer HAS2

<220>
<221> misc feature
<222> (1)..(24)

<400> 11
tctgacagca cagaatccac tgcc 24

<210> 12
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer
HAS3-A-tail

<220>
<221> misc feature
<222> (1)..(22)

<400> 12
ttgactgctg attttattgc ag 22

<210> 13
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: primer HS1

63

<220>

<221> misc feature

<222> (1)..(22)

<400> 13

gctttccagt ctggtggtga at 22

<210> 14

<211> 455

<212> PRT

<213> Homo sapiens

<220>

<221> SITE

<222> (1)..(455)

<223> /note="Human AMCase"

<400> 14

Tyr	Gln	Leu	Thr	Cys	Tyr	Phe	Thr	Asn	Trp	Ala	Gln	Tyr	Arg	Pro	Gly
1				5					10					15	

Leu	Gly	Arg	Phe	Met	Pro	Asp	Asn	Ile	Asp	Pro	Cys	Leu	Cys	Thr	His
				20				25						30	

Leu	Ile	Tyr	Ala	Phe	Ala	Gly	Arg	Gln	Asn	Asn	Glu	Ile	Thr	Thr	Ile
		35					40					45			

Glu	Trp	Asn	Asp	Val	Thr	Leu	Tyr	Gln	Ala	Phe	Asn	Gly	Leu	Lys	Asn
	50						55					60			

Lys	Asn	Ser	Gln	Leu	Lys	Thr	Leu	Leu	Ala	Ile	Gly	Gly	Trp	Asn	Phe
	65				70					75					80

Gly	Thr	Ala	Pro	Phe	Thr	Ala	Met	Val	Ser	Thr	Pro	Glu	Asn	Arg	Gln
				85					90						95

Thr	Phe	Ile	Thr	Ser	Val	Ile	Lys	Phe	Leu	Arg	Gln	Tyr	Glu	Phe	Asp
			100						105					110	

Gly	Leu	Asp	Phe	Asp	Trp	Glu	Tyr	Pro	Gly	Ser	Arg	Gly	Ser	Pro	Pro
		115					120					125			

Gln	Asp	Lys	His	Leu	Phe	Thr	Val	Leu	Val	Gln	Glu	Met	Arg	Glu	Ala
		130				135						140			

Phe	Glu	Gln	Glu	Ala	Lys	Gln	Ile	Asn	Lys	Pro	Arg	Leu	Met	Val	Thr
	145				150					155					160

Ala	Ala	Val	Ala	Ala	Gly	Ile	Ser	Asn	Ile	Gln	Ser	Gly	Tyr	Glu	Ile
					165				170					175	

Pro	Gln	Leu	Ser	Gln	Tyr	Leu	Asp	Tyr	Ile	His	Val	Met	Thr	Tyr	Asp
			180					185						190	

Leu	His	Gly	Ser	Trp	Glu	Gly	Tyr	Thr	Gly	Glu	Asn	Ser	Pro	Leu	Tyr
		195					200						205		

Lys Tyr Pro Thr Asp Thr Gly Ser Asn Ala Tyr Leu Asn Val Asp Tyr			
210	215	220	
Val Met Asn Tyr Trp Lys Asp Asn Gly Ala Pro Ala Glu Lys Leu Ile			
225	230	235	240
Val Gly Phe Pro Thr Tyr Gly His Asn Phe Ile Leu Ser Asn Pro Ser			
245	250	255	
Asn Thr Gly Ile Gly Ala Pro Thr Ser Gly Ala Gly Pro Ala Gly Pro			
260	265	270	
Tyr Ala Lys Glu Ser Gly Ile Trp Ala Tyr Tyr Glu Ile Cys Thr Phe			
275	280	285	
Leu Lys Asn Gly Ala Thr Gln Gly Trp Asp Ala Pro Gln Glu Val Pro			
290	295	300	
Tyr Ala Tyr Gln Gly Asn Val Trp Val Gly Tyr Asp Asn Ile Lys Ser			
305	310	315	320
Phe Asp Ile Lys Ala Gln Trp Leu Lys His Asn Lys Phe Gly Gly Ala			
325	330	335	
Met Val Trp Ala Ile Asp Leu Asp Asp Phe Thr Gly Thr Phe Cys Asn			
340	345	350	
Gln Gly Lys Phe Pro Leu Ile Ser Thr Leu Lys Lys Ala Leu Gly Leu			
355	360	365	
Gln Ser Ala Ser Cys Thr Ala Pro Ala Gln Pro Ile Glu Pro Ile Thr			
370	375	380	
Ala Ala Pro Ser Gly Ser Gly Asn Gly Ser Gly Ser Ser Ser Ser Gly			
385	390	395	400
Gly Ser Ser Gly Gly Ser Gly Phe Cys Ala Val Arg Ala Asn Gly Leu			
405	410	415	
Tyr Pro Val Ala Asn Asn Arg Asn Ala Phe Trp His Cys Val Asn Gly			
420	425	430	
Val Thr Tyr Gln Gln Asn Cys Gln Ala Gly Leu Val Phe Asp Thr Ser			
435	440	445	
Cys Asp Cys Cys Asn Trp Ala			
450	455		

ABSTRACT

[0139] The invention provides a mammalian mucinase capable of hydrolyzing mucin. The mucinase is among others suitable for counteracting diseases in which mucus is involved. The diseases comprise cystic fibrosis, COPD, asthma, bronchitis, tuberculosis, tumors with altered mucus expression, and mucus-containing pathogens. The invention also provides a pharmaceutical composition comprising an effective amount of the mucinase and a method of therapeutic or prophylactic treatment of an individual against a disease in which mucus is involved. Methods for obtaining the mucinase are also herewith provided, as well as nucleic acids encoding (part of) the mucinase. In one aspect, the invention provides a diagnostic kit comprising a mucinase, a mucinase-specific antibody, a mucinase-derived peptide and/or nucleic acid encoding (part of) the mucinase.